ON THE MECHANISM OF MORPHOLOGICAL DIFFERENTIATION IN THE NERVOUS SYSTEM

II. THE RELATION BETWEEN COMPRESSION AND THE DEVELOPMENT OF A SERIES OF VESICLES.

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EIGHT TEXT FIGURES AND THREE PLATES

I. INTRODUCTION

The simple neural tube mentioned so frequently in embryological literature is really no more than a convenient fiction. Practically it cannot be found at any stage of development. The mere differences in the amounts of tissue in the anterior and posterior ends of the neural plate alone preclude this possibility, but in addition, long before the separation of the plate from its extra-neural ectoderm is complete, the developing 'tube' prefigures a serial differentiation that culminates in a succession of vesicles, within limits, highly constant for the vertebrate nervous system, and, as one of its basic attributes, calling for explanation.

The stages in embryogenesis upon which a study of this problem must be based, are necessarily as commonplace as those dealt with in my first paper[1] yet despite their familiarity with this period of development, embryologists do not tell us why the nervous system differentiates a series of vesicles, and particularly why there are five such fundamental divisions in its anterior end. Many do not even ask the necessary questions although His, over forty years ago, considered the subject as legitimately within the province of the student of development.

[1] On the Mechanism of Morphological Differentiation in the Nervous System. 1. The Transformation of a Neural Plate into a Neural Tube. Anat. Rec., vol. 8, pp. 525–551, 1914.

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In fact His[2] himself attempted to explain serial differentiation. The analysis, in Unsere Körperform, begins with the 'closed neural tube,' whose anterior end, as indicated in figure 1, is larger than its posterior, and is characterized by three enlargements—the precursors of the five secondary brain vesicles.

In a later stage, figure 2, His illustrates the relative positions of the five secondary vesicles in the flexed state. The curve, typical of cranial flexure, is divided into a 'Brückenkrümmung,' involving the fifth and fourth vesicles; a 'Mittelwölbung,' involving the mid brain; and the 'Hackenkrümmung,' involving the second and first. At the extreme anterior point of the first vesicle is the 'Trichterfortsatz' Tr.

His then attempts to show how this form can be derived from that given in figure 1. The brain, according to the argument, begins as a tube whose comparatively large lumen is enclosed by moderately elastic walls. These conditions are identical with those given in a piece of rubber tubing.

If the edge of a rubber tube is made fast by threads in the manner indicated in figure 3 longitudinal compression will pro-

Fig. 1 After His. Chick embryo of the second day. *H*, brain, with three vesicles indicated; *Ag*, optic vesicle; *W*, Wolffian ridge; *Uw*, somites; *Ump*, unsegmented mesodermal bands; *Am1*, head fold of the Amnion; *Am2*, lateral folds of the Amnion; *Mp*, open portion of the neural tube. The heart is indicated by means of dotted lines. Enlarged 20 ×.

Fig. 2 After His. Brain of chick embryo of the third day. *Ag*, optic vesicle; *Vh*, forebrain; *Tr*, 'Trichterfortsatz;' *Zh*, interbrain; *Mh*, midbrain; *Hh*, hindbrain; *R*, location of fourth ventricle; *Br*, 'Brückenkrümmung;' *Nh*, afterbrain; *Gh*, auditory vesicle. Enlarged 30 ×.

Fig. 3 After His. Rubber tube whose upper end has been drawn backwards by means of a thread.

Fig. 4 After His. For explanation of legends see figure 2. The dotted line indicates the anterior limits of the foregut.

Fig. 5 Sagittal section through chick-head about 38 hours old, showing the relations of foregut, floor of the second brain vesicle, and the anterior end of the notochord.

Fig. 6 After His. Upper figure, head of embryonic pike; middle, of trout, and lower, dorsal view of same. Legends as in figure 2. *Rg*, olfactory pit.

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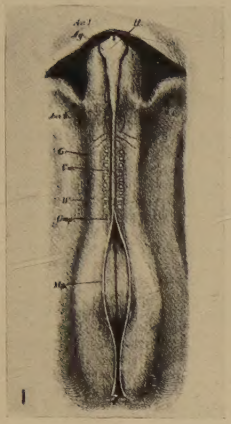
[2] Wilhelm His: Unsere Körperform und das Physiologische Problem ihrer Entstehung. F. C. W. Vogel, Leipzig, 1874. Achter Brief, pp. 93–104.



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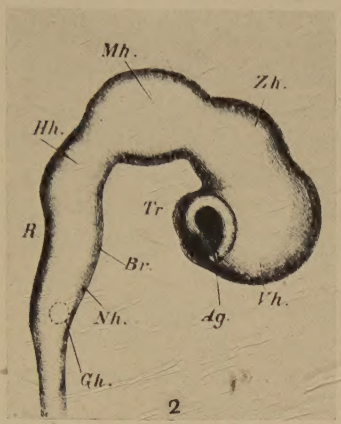

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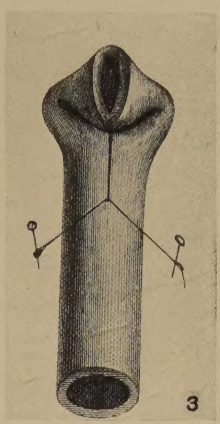

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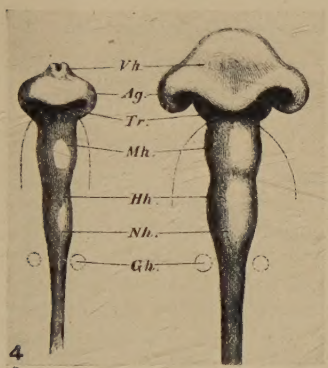

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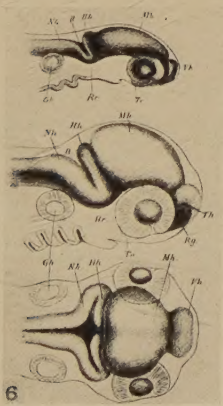

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duce an abrupt bend just behind the level of fixation, and there will appear in this region a pair of lateral swellings, whereas on one side—either the upper or the lower, as the case may be,—a sharp groove, deepest in the median line, shallower toward the edges of the sidewise expansions, will cross the tube in a transverse curve with concave face forward.

His points out how exactly this form duplicates the ventral surface of the second vesicle in the stage of development depicted in figure 1.

The suggestion that identity of form in the two sets of cases is the result of identity in the mechanical conditions under which they were produced is very much strengthened, as His emphasized, by the union "welche durch den Axenstrang und später durch die aus ihm entstandene chorda dorsalis zwischen der Medullarplatte und dem Darmdrüsenblatte, längs der Mittellinie unterhalten wird."[3]

This union later undergoes the following modifications: first the entoderm separates from the chord,

> und, viel später, diese vom Medullarrohre . . . . Am innigsten ist die Verbindung durch zwischengelagerte Masse zwischen dem Ursprünglich vordersten Rande der Medullarplatte und vom vorderen Ende des Vorderdarmes. Die Verbindung ist hier eine so innige, dass, wenn in sehr später Zeit der Vorderdarm vom Gehirn sich trennt, die Trennung nicht im Verbindungsstücke geschieht, sondern in der Continuität des Vorderdarmes selbst. Ein kleines stück von diesem bleibt als Vorderer Lappen der Hypophysis in dauernder Verbindung mit dem Gehirn.[4]

His continues,

> Es wächst aber das Medullarrohr, und speciell das Gehirn rascher in die länge als der Vorderdarm; da es nicht zu einer Trennung beider Theile kommt, so muss der längere Theil sich Krümmen, und müssen ferner die unmittelbaren Folgen der Zerrung in den mit einander verbundenen Strecken des Vorderdarms sowohl, als des Medullarrohres zu Tage treten. Beides trifft in sehr prägnanter Weise ein, nicht allein erhebt sich das Medullarrohr über dem Vorderdarm in waschendem Bogen, sondern es ziehen sich an beiden Theilen die Verbundenen Enden trichterförmig aus, wir bekommen auf die Weise am

[3] Loc cit., p. 99.
[4] Loc. cit., p. 100.

Gehirn den oben betrachteten Trichterfortsatz (fig. 2), am Vordarm die . . . . bekannte sog. Rathke'sche Tasche.[5]

The union of the 'trichterfortsatz' with Rathke's pouch is, under the conditions of growth, mechanically the exact equivalent of the piece of string in figure 3. The striking similarity of the forms produced in the two cases scarcely requires comment and may almost be considered proof of the correctness of the assumptions. One factor, not emphasized by His, however, is the notochord. Given the fusion between 'Trichterfortsatz' and Rathke's pouch, flexure resulting from the faster growth of the nervous system would be accentuated by the notochord whose anterior end would play the rôle of a fulcrum about which the curvature would take place.

But His' account is not free from anachronisms, nor will it bear a too close scrutiny from the standpoint of comparative anatomy and embryology. The final conclusion which he wishes drawn, "von der Abhängigkeit . . . . in welcher die Gehirngliederung von den auftretenden Longitudinalbiegungen des Organs steht,"[6] does not follow. If this were correct, sharpness in the demarcation of the vesicles from one another should vary directly with the degree of cranial flexure in individual as well as comparative ontogeny. This, for the first case is not strictly true, and, for the second, scarcely at all, since the vesicles are distinct before cranial flexure, and forms exhibiting a high degree of flexure have their vesicles no more sharply delimited than those in which the bending of the embryonic head is never very marked. I have no reason to doubt that His was correct with respect to the origin of both the flexure, and the lateral expansions, or optic lobes, but the differentiation of the vesicles themselves cannot be explained as the result of flexure.

II. ON THE EFFECTS OF DIFFERENTIAL GROWTH

It is clear that His considered differential growth the cause of flexure, and flexure the cause of vesiculation. Since, however, only the first vesicle with its ventral groove and lateral expan-

[5] Loc. cit., p. 100.
[6] Loc. cit., p. 104.

sions can be accounted for by flexure, the case for this factor is hardly made out.

From his manipulation of rubber models, forced in various ways to simulate special regions of the nervous system, as well as from the general tenor of the argument, it is likely that His would not have denied that differential growth, in the early stages of flexure, results in some sort of compression in the longitudinal axis. Be this as it may, it is certain that he attributed to this factor not only flexure, but also the changes in relative position which the vesicles undergo during later stages of development. Concerning the head of the embryonic pike, figure 6, he writes: "Durch die wachsende Zusammenschiebung der Theile ist der hintere Hirnabschnitt, oder das Nachhirn unter die davor liegende Anlage des Kleinhirns, und diese unter diejenige des Mittelhirns geschoben worden."[7]

III. HIS' DEMONSTRATION OF DIFFERENTIAL GROWTH

His attempted to demonstrate the differential growth of the nervous system on chick embryos ranging roughly from the twenty-fourth to the ninety-sixth hour of incubation.[8] The method consisted in comparing at four levels, those of the eye, the 'Gehirnblase,' and the first pair of somites, the areas in transverse section of the 'neural tube'—thought of as spread out flat, and the ectoderm, measured from the median axis to the point of fusion with the lateral muscle plate. On this basis, he was able to convince himself that the nervous system actually grows at a faster rate than the tissues with which it was compared.

Although this method may be capable of demonstrating the point, it is certainly not able to show that differential growth in width is accompanied by differential growth in length. The

[7] Loc. cit., p. 103.

[8] According to his own statements, the youngest stage used was of the second day, but his figure with its nine pairs of somites hardly bears this out. See figure 1 of the present paper.

association of the two is of course very probable, but by no means necessary.

Differential growth in length can be determined directly only by measurements in the long axis, and if the values so secured have a certain sense and magnitude, they may be made the basis for inferences concerning compression in that axis.

IV. METHOD OF DETERMINING COMPRESSION IN THE LONG AXIS

In order to discover the presence or absence of compression in the long axis it is necessary to find at least one measurable relation which differential growth either changes or brings about. This relation must be longitudinally effective, widely applicable, in magnitude independent of absolute measurements, and finally, capable of exact expression.

Many attempts were made to satisfy these conditions before it was found that the necessary data, accuracy, and reliability were obtainable in a very simple way.

With the camera lucida, optical sections of embryonic chick heads, at convenient magnification and a focus giving maximal outlines, were carefully traced. About these outlines I then erected the system of lines indicated in figure 7.

The base of this system is a line tangential to the anterior face of the first pair of somites. Upon this base perpendiculars, themselves tangential to the sides of the head at its widest level, were erected, and finally, parallel with the somitic base line, a tangent to the anterior edge of the head. In this way the entire cephalic region is included within the area of a rectangle.

The purpose of these preliminaries is to get a measure of the length of the head. Without the rectangle it would be easy enough to find an anterior point of reference, but it is never possible to tell exactly where the posterior limit of the head is. In fact in the early stages this grades so insensibly into the body that any posterior limit permitting comparison between various embryos and different stages, of necessity has to be chosen arbitrarily. If then an arbitrary point is imposed by the conditions of the case, it is best to choose one whose relations to the rest

of the embryo are not only significant, but also likely to present the greatest relative constancy. The somites possess these qualifications more than any other structures, and so I took the longer sides of the cephalic rectangle which rests upon the first pair, not as *the*, but as *a* measure of head length.[9] Since we desire a measure of differences in the rate of linear growth be-

Fig. 7 Diagram to illustrate the cephalic rectangle. The line tangential to the anterior face of the first pair of somites, which are indicated in solid black, is the somitic base line. The perpendiculars erected upon this line are tangential to the head at its widest points. The head-length was arbitrarily determined along these perpendiculars as the distance between the somitic base line and the parallel tangent to the anterior surface of the head.

[9] To carry out these and the subsequent measurements on live embryos presents, for the present, insuperable difficulties. Fixed material, no doubt, differs in absolute values from the living, and it is possible that the relations between measurements which I shall discuss, are also not the same. However, absolute values need not concern us at all in the present connection, and if the relative values upon which my argument rests were seriously changed, I should hardly expect the constancy in sense which they exhibit.

tween the neural mass and the head, the simplest method is to compare the length of the head with the length of the nervous system, in various stages of development, measured from the somitic tangent as a base. Provided the head of the embryo exhibits no serious irregularities, its length may be determined arbitrarily in the manner indicated above, but, on account of the vesicles, the length of the 'brain' cannot be given by the cephalic rectangle.

Several methods involving the volume of the brain were tried but discarded in favor of one which in addition to simplicity, is capable of giving exactly and directly the very information that is wanted. All that is necessary is to determine by means of a map-measurer the perimeter of the nervous system beginning at its intersection with the somitic tangent on one side and ending at the corresponding point on the other. The length of the nervous system will be half this perimeter.[10]

V. THE NEURO-CEPHALIC QUOTIENT

With the aid of these measurements it is possible to express in the form of a fraction the relation in each stage between the length of the head and that of the nervous system. The fraction chosen is derived by dividing half the neural perimeter into the head length and tells us how many units of head length, within the limits of the cephalic rectangle, are available for every unit of length in the nervous system. This fraction I shall call the Neuro-Cephalic Quotient.

Very few embryos approach the ideals of rectitude and symmetry depicted in text-books and wall-charts. Out of the entire collection with which I have worked I have been able to find only eight justly to be characterized as diagrammatic. The outlines of these are reproduced in plate 1, figures A, B, C, D, E, F, G, and H, whereas the number of somites, and the corresponding neuro-cephalic quotients are given in table 1.

[10] Since this method is applicable to the nervous system it might be asked why I did not apply it also to the head instead of relying on the long sides of the cephalic rectangle. The answer is, because the perimeter of the head in early stages is distinct only in the anterior region.

On account of the small number of cases the scientific value of this particular table is negligible. Nevertheless it suggests certain problems and questions which must be dealt with before we can arrive at a just estimate of the significance of the quotient.

The values, taken for the time being as they stand, seem to indicate that when diagrammatic embryos are arranged in series according to somites—or, in other words, according to age and degree of development, the size of the quotient will be found to vary inversely with the number of somitic units. This, if correct, means that as development goes on the amount of head length into which a given length of nervous system must fit, decreases progressively.

TABLE 1

EMBRYO	NUMBER OF SOMITES	QUOTIENT
A	2	1.485
B	8	0.902
C	9	0.877
D	9	0.837
E	10	0.820
F	10	0.820
G	10	0.846
H	11	0.772

Given this condition, differentiations of some sort, spreading, collapse, telescoping, or vesiculation, are to be expected. Leaving aside, for the moment, the question why vesiculation prevails, let me explain why I choose to consider first these rare diagrammatic forms. The reason is very simple: because in these, the presence of a high degree of symmetry indicates that the developmental processes in general have been in nearly perfect balance, and have given a result relatively or quite free from complications calling for special explanations. Briefly, these forms are the simplest available.

Granted the advantage of simplicity—we must ask why a well balanced development gives rise to the obvious discrepancies which the table exhibits and furthermore why it is itself so rare.

The effect of error introduced by the various technical methods employed in preparing the embryos for study cannot be denied,

nor can I claim for my measurements a maximal degree of accuracy. Nevertheless I see no reason for suspecting greater errors than inhere in embryological and biometric work in general. For reasons which I shall attempt to make clear, I believe that the major discrepancies inhere in the material itself.

Referring to the table, the general sense of the values is very obvious. There is, however, no absolute proportionality between somitic increase and the shrinking quotient. Furthermore, embryos like C and D, each with nine somites, have quotients separated by a wider margin than C and G. A series arranged according to somites, even in diagrammatic forms, does not coincide absolutely with a series based on quotients. That there is a general coincidence, however, will hardly be denied.

It was the desire to find embryos in which just this sort of discrepancy could be expected to assert itself in minimal degree that lead me to select the most diagrammatic forms for separate treatment. But even in these complete elimination of discords is impossible. If now the lack of harmony between theory and practice is to be sought in the embryo itself rather than in the methods by which it was handled, we must analyze our material in an attempt to get at the real explanation.

Such explanation will be found, I think, when we realize the true nature of the developmental process itself. To be alive is to solve a constellation of interlocking problems in equilibration. In the adult, departures from balance occur within comparatively restricted compass, and, being for the most part quickly reversed, result in few or relatively unimportant morphogenetic changes;[11] in the embryo, on the contrary, the excursions are often very wide. Indeed it is hardly too much to say that a developing organism 'blunders' from one crisis to another, until gradually, by the narrowing of its 'horizon,' it reaches that state of relative stability which is characteristic of the adult. Nothing that happens in the fully developed organism can be compared with the multiplicity and complexity of the immediate and remote adjustments consequent upon the differentiation of

[11] See Glaser, The Basis of Individuality in Organisms. Science, vol. 44, pp. 219–224.

the germ layers From the dynamic standpoint, development might be defined as the symptom of an organic instability in which departures from exact balance occur within limits so wide as to escape fatality by only a narrow margin.

This granted, it follows that the sum of opportunities within the developing system is exceedingly great. Although unquestionably exact and theoretically predictable in all its details, embryogenesis, within the boundaries of what is 'normal,' nevertheless, varies tremendously. It need occasion no surprise then if we find differences in the several tissue-maneouvres, or in the exact time of onset of this, that, or the other process. Of several embryos which might be expected to exhibit identical conditions in all respects, one may lead or lag in the morphogenetics of its nervous system, a second in that of its somites, and another in its circulatory equipment. In fact the early discrepancies or temporary misfits of development may at any instant simulate disorganization. By this it is not intended to suggest that 'normal' embryogenesis is strictly a matter of chance, but only that its 'administration' appears relatively loose. To us this looseness is emphasized subjectively because we remain ignorant of so large a share of the elements underlying the process. We may be sure, however, that the minor errors of development sooner or later receive adequate correction for the end results of embryogenesis are precise and give an organism which actually describes the genetic constitution of its ancestors.[12]

Taking development as it is, our quotient, to have significance, must be applicable to a wide range of cases, which, no matter how they may deviate from the diagrammatic, nevertheless cannot be considered otherwise than normal. Within this range, if our preliminary test is to be trusted, we should find the same general relations exhibited by the ideal embryos. However, since the measurements upon which the quotient rests, themselves bear no obviously immediate relation to the factors upon which the production of somites depends, we should not expect absolute correspondence between somitic increase and a falling

[12] See Note 11.

quotient. All that we are entitled to expect is that the quotient in general will vary inversely with the number of somites.

If the quotient is a true indicator of compression in the longitudinal axis, and if compression is related causally to serial differentiation, we are also entitled to expect some relation between this differentiation and the quotient. Here again, we should not set our minds on absolute correspondence, for quite apart from the difficulty of exact determinations in this connection, the amount of differentiation which a given degree of compression calls forth depends on many things. Age is one; what differentiations had taken place before a particular degree of compression was reached, is a second; the thickness and mechanical properties of the nervous system are a third; the rate at which the differentiations are produced is a fourth; the exact axis of maximal compression is a fifth; and no doubt there are many others. There is, however, no accurate way, particularly in the more complicated cases, of expressing the degree of differentiation. Furthermore the attempt to do so would necessitate also the consideration of that portion of the system which lies posterior to the somitic base line of the cephalic rectangle. Nevertheless, little serial differentiation should be associated with a large, and the reverse with a small quotient.

Correspondences between quotient and somites on the one hand, and quotients and differentiation on the other, do not complete the list of what we may expect. A third matter—that of aberrancies—must receive consideration.

From our present standpoint, the aberrancies theoretically to be considered are those in which the nervous system is either underdifferentiated or has erred or has been forced to err seriously in the opposite direction. In the first case we should expect relatively high, in the second, relatively low, quotients. As a matter of fact, I have not found enough cases of undifferentiation to feel warranted in giving them special prominence, but overdifferentiation is common. In fact, in one of its varieties, it is too common to leave much doubt that the resulting forms must be classified as normal. This type, characterized by the partial telescoping of the first and second vesicles exceeds in

frequency the known percentage of faulty hatchings or total failures by so large a margin as to suggest very strikingly indeed the likelihood that many nervous systems during some stage of their development exhibit temporarily, at least, too much differentiation. Cases in point are illustrated on plate 2, embryos I, J, K, L, M, N, O.

To what extent now, our several expectations are fulfilled can be seen by comparing the figures of the forms illustrated in plates 1 and 2, with the corresponding values given in table 2 in which the embryos are arranged in a series beginning with the highest and ending with the lowest quotient.

The omission from this table of 12 and 14 somite embryos is due to the fact that only one of each kind was available. The only other grounds of elimination were asymmetry or a degree of aberrancy which could have but one interpretation. Such embryos with somites and quotients indicated are referred to in table 3 and illustrated in plate 3.

If we accept table 2 as indicative of the norm for the various stages dealt with, the quotients in table 3 become significant. As can be seen by reference to plate 3, the embryos now under consideration fall into two groups—in one of these, including U, W, and X, the embryo is asymmetrical, in the other, T, V, Y, and Z, the embryos are overdifferentiated. With respect to Z, nothing definite can be said, since table 2 does not contain the quotient normal for the 27 somite stage. Embryos T, V, and Y, however, all have quotients too low for their respective ages. In other words the compression to which the nervous system is subjected is higher than normal and has resulted in too much differentiation.

The asymmetrical forms, U, W, and X, suggest that if a certain degree of compression fails to set in, or is not properly directed, the aberrant condition will be reflected in an asymmetrical distribution of the neural mass.

CONCLUSION

A comparative study of the illustrations in the plates and the values given in tables 1, 2, and 3 may, I think, be justly summarized by saying that our expectations in general have been

TABLE 2

Normal embryos

QUOTIENT	SOMITES	REMARKS
1.890	3	
1.672	2	
1.485	2	
1.313	4	
1.132	3	
1.0475	5	
1.017	5	
1.012	4	
1.0095	5	
0.968	6	
0.952	6	
0.949	6	
0.925	8	
0.920	7	
0.911	10	
0.903	7	
0.902	8	
0.9009	9	
0.887	9	
0.877	9	
0.871	10	
0.863	9	
0.851	10	
0.849	10	
0.847	10	
0.846	10	
0.842	10	
0.842	10	
0.837	9	
0.820	10	
0.820	10	
0.818	11	
0.812	11	
0.811	10	
0.800	11	
0.772	11	
0.737	13	Telescoped forms Plate 2 I
0.664	13	Telescoped forms Plate 2 J
0.649	15	Telescoped forms Plate 2 K
0.605	16	Telescoped forms Plate 2 L
0.592	15	Telescoped forms Plate 2 M
0.577	16	Telescoped forms Plate 2 N
0.573	17	Telescoped forms Plate 2 O
0.572	17	
0.572	16	

TABLE 3

QUOTIENT	EMBRYO	SOMITES	REMARKS	QUOTIENT
0.817	T	9	Asymmetrical	Too low
0.790	U	13	Asymmetrical	Too high
0.789	V	10	Overdifferentiated	Too low
0.787	W	13	Asymmetrical	Too high
0.768	X	13	Asymmetrical	Too high
0.541	Y	14	Overdifferentiated	Too low
0.522	Z	27	Overdifferentiated	

fulfilled. The relations postulated in advance between the neurocephalic quotients, on the one hand, and aberrancies, degrees of normal differentiation, and somitic increase on the other, are capable of being verified. The relation which at this time I wish to especially emphasize, is the association of a falling quotient with the multiplication of somites.

The quotient correctly understood is a measure of longitudinal compression. The somites are recognized as a measure of development. It follows, therefore, that the movements of the quotient are related in time and sense to the serial differentiation to the nervous system precisely in the manner in which they should be related, if compression in the longitudinal axis is a condition upon which the vesiculation of the nervous system

TABLE 4

SOMITES	CASES	QUOTIENT
2	2	1.579
3	2	1.511
4	2	1.163
5	3	1.025
6	3	0.956
7	2	0.912
8	2	0.914
9	5	0.873
10	11	0.845
11	4	0.801
13	2	0.701
15	2	0.621
16	3	0.585
17	2	0.573

depends. The assumption of a 'causal' connection between compression and the formation of the brain vesicles is therefore warranted.

The justice of this assumption appears still greater when we average the results for each stage of development dealt with in table 2. This was done and the outcome is given in table 4.

If now, with the aid of table 4 we construct a curve in which quotients are plotted along the ordinate and the number of somites along the abscissa, the relation between a falling quo-

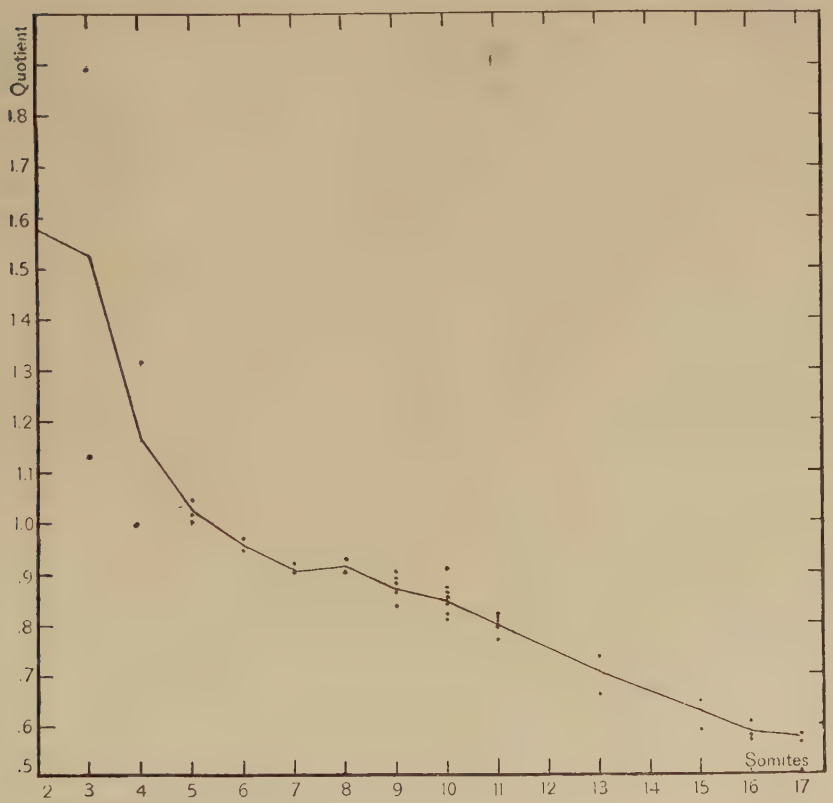


Fig. 8 Showing the relation between a falling neuro-cephalic quotient and somitic increase. The curve is constructed by joining the loci of the averages given in table 4. The dots indicate the loci of the individual quotients given in table 2.

tient and somitic increase exhibits itself in the most striking manner.

On the basis of this curve, figure 8, I infer that during the period of development considered, a rising state of longitudinal compression is one of the conditions determining the differentiation of a series of vesicles in the brain.

SUMMARY

1. Cranial flexure, although capable of explaining the lateral and ventral differentiations of the prospective second brain vesicle, is nevertheless not related to the general process of vesiculation in the manner in which it should be if the formation of vesicles were dependent upon flexure, as His maintained.

2. According to His, flexure depends on differential growth.

3. According to the results presented in the present paper, vesiculation also depends upon differential growth and precedes flexure.

4. Differential growth, to play a rôle in this connection, must be longitudinally effective. Such effectiveness is undemonstrable by the method of His.

5. Effectiveness in the long axis can be demonstrated by comparing the length of the embryonic head with that of the nervous system. The relation between these two measurements, within the arbitrary limits set by the cephalic rectangle, has been expressed in the form of a fraction.

6. This fraction, the Neuro-Cephalic Quotient, is derived by dividing half the perimeter of the nervous system into the head-length. It tells how many units of head-length are available for every unit of length in the nervous system.

7. The quotient is largest in the earliest stages, and decreases with the progress of development. It is inversely proportional to the number of somites, and, so far as can be determined in the absence of accurate modes of expression, with the degrees of differentiation exhibited by the nervous system. Telescoped forms, and those abnormally over-differentiated, have expectedly low quotients.

8. Despite the variability which inheres in developmental processes by their very nature, the relations between quotient on the one hand, and somites, or differentiation of the nervous system, on the other, are such as to warrant the conclusion that a rising state of compression in the longitudinal axis is one of the important conditions under which the vesiculation of the embryonic brain takes place.

PLATE 1

EXPLANATION OF FIGURES

Outlines of heads of diagrammatic 2 to 11 somite chick embryos referred to in table 1. The posterior line through the nervous system is the somitic base line. In embryo A the nervous system did not extend backward as far as the somitic tangent. In this case two base levels are indicated, one used to measure head-length, the other to determine the neural perimeter.

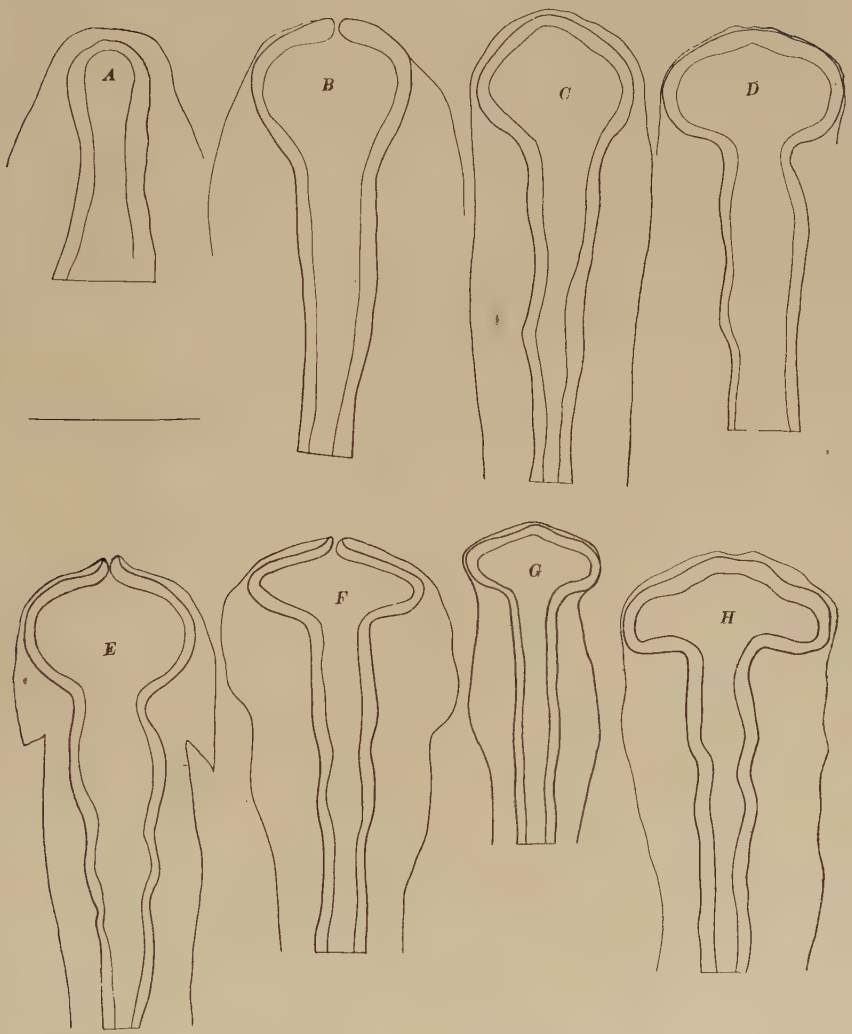

A
B
C
D
E
F
G
H

PLATE 2

EXPLANATION OF FIGURES

Telescoped forms with 13 to 17 somites and low quotients. Referred to in table 2.

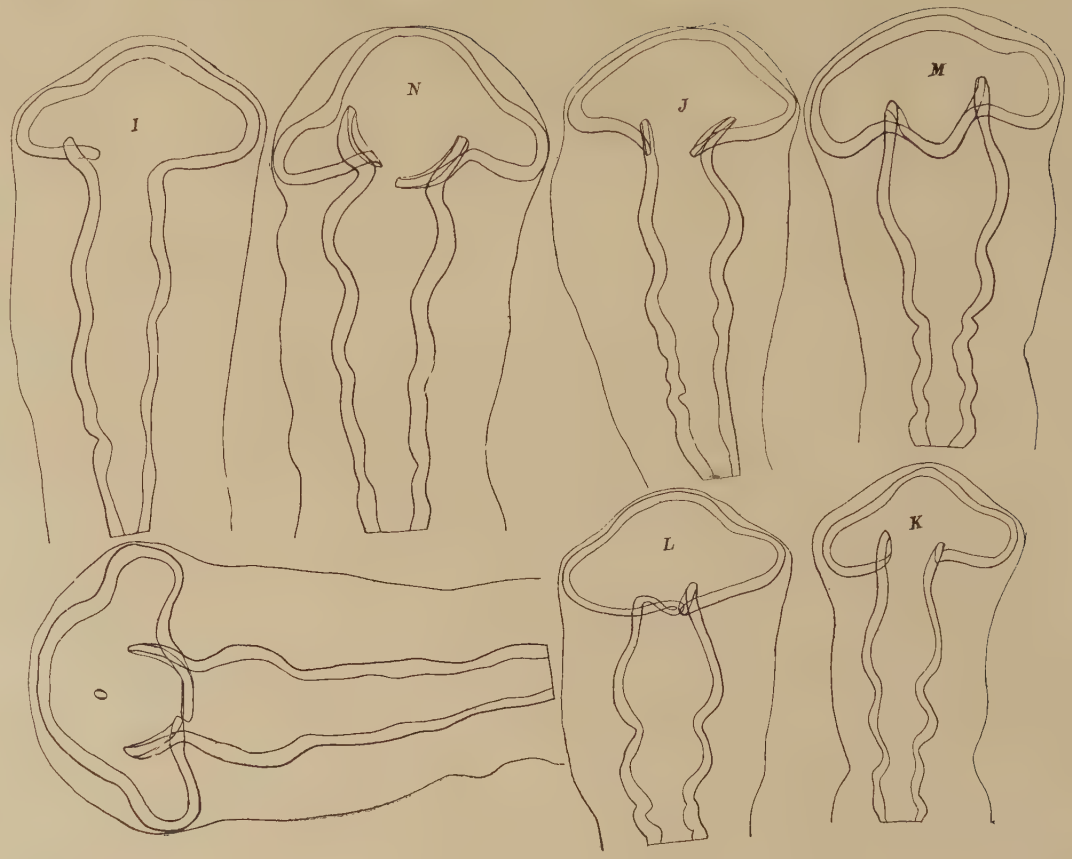
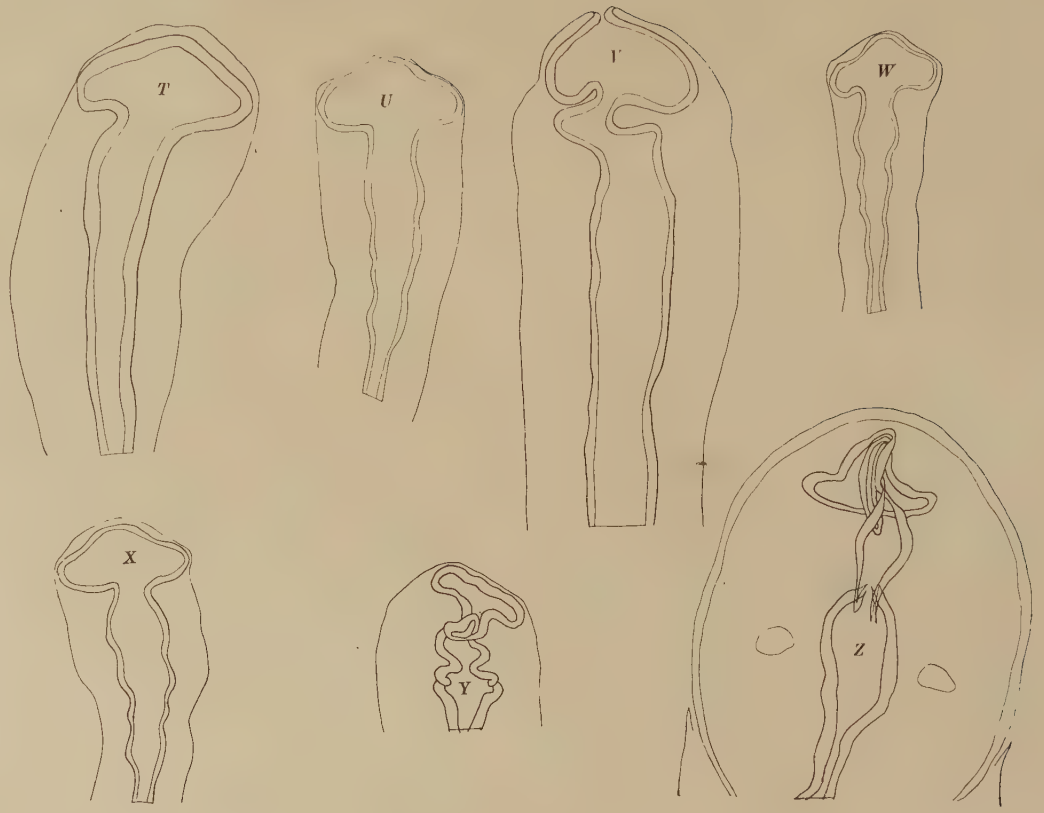

I
N
J
M
L
K
O

PLATE 3

EXPLANATION OF FIGURES

Aberrant forms. Embryos T, V, Y, and Z are over-differentiated. As shown in tables 2 and 3, T, V, and Y have quotients too low for their respective ages. The quotient normal for the 27 somite stages has not been determined; therefore no statement can be made regarding embryo Z.

Embryos U, W, and X, have quotients too high for their respective ages, and suggest that compression must reach a certain magnitude and moreover be properly directed in order that the neural mass may distribute itself symmetrically.


T
U
V
W
X
Y
Z

THE CHOROID PLEXUS WITH SPECIAL REFERENCE TO INTERSTITIAL GRANULAR CELLS

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TEN FIGURES

During a comparative study of the structure of the choroid plexus in various mammals, in association with Dr. Harvey Cushing at the Hunterian Laboratory, Johns Hopkins University, I observed the presence of very prominent interstitial granular cells in the choroid plexus of the ox. They are especially numerous in the ox and are present to a less degree in the sheep and swine. I have failed to find any reference to these cells in the literature on the choroid plexus, and it is primarily to report them that this paper is submitted. I have included, as well, my observations on the structure of the epithelial layer of cells, in view of their now generally accepted connection with the secretion of cerebro-spinal fluid.

GENERAL ARCHITECTURE

The choroid plexus of the ox is similar in structure to that of other mammals, as generally described. Tufts of blood vessels varying in size and thickness of the connective tissue coats are seen covered by a single layer of cuboidal cells.

The various elements making up the choroid plexus are clearly differentiated in tissues fixed in formalin-bichromate solution and stained in Van Gieson's. The yellowish-brown stained surface cuboidal cells are sharply demarcated from the red stained connective tissue which surrounds the blood vessels. In some tufts the connective tissue coats which form the walls of the blood vessels measure 0.2 mm. in thickness, while in others they form

only a thin membrane between the vessel lumina and the surface cuboidal cells. Much variation exists also in the size of the lumina (fig. 1).

Generally speaking, the vessels of the choroid plexus may be divided into two groups: 1) The vessels with the thick walls possess as a rule narrow lumina and have the same structure in general as do small arteries. Weigert's stain shows a well developed tunica elastica interna, which in most instances is cor-

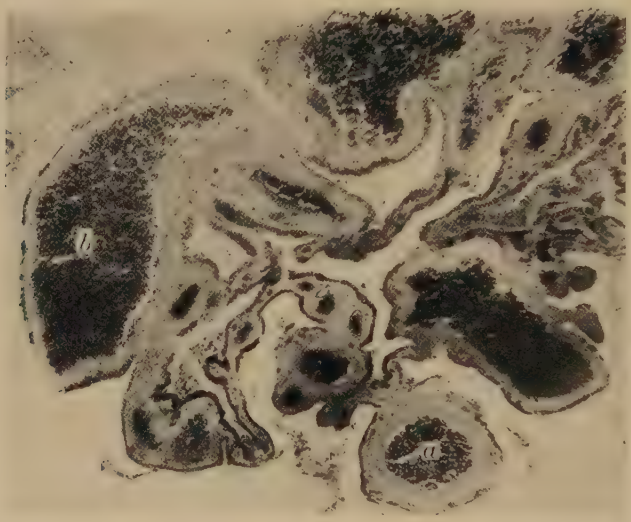

Fig. 1 Choroid plexus of the ox. *a*, artery; *b*, vein or sinus. Microphotograph, oc. 10, obj. 16 mm., B. & L. Tech: Bensley's alcohol bichloride bichromate solution, haematoxylin and eosin.

rugated. Fine, waving elastic fibers may be seen in the tunica media, which is well developed. The fibers may be further traced to the cuboidal cells and are frequently seen to form a basement membrane for these cells. 2) The second group of vessels are those with comparatively thin walls and wide lumina. These may be regarded as veins or sinuses. The walls are composed almost entirely of white fibrous connective tissue and cannot be differentiated into intima, media, and adventia, but are similar in structure throughout. Only here and there are

seen fine elastic fibers. No muscular walls can be made out in this second group.

The walls contain numerous small vessels and capillaries (Vasa vasorum) of varying calibres. They are rich also in nuclear elements, such as endothelial cells, connective tissue cells and interstitial granule cells (fig. 1). A discussion of these interstitial granule cells follows that of the cuboidal surface cells.

EPITHELIAL CELLS

The epithelium forms a single layer of cuboidal cells. This observation agrees with those of Meek (’07) and others who describe a single layer of cells. Luschka (’55), Findlay (’89) and Kölliker (’96), however, state that several layers are present. The cells on some tufts are more or less flattened while on other tufts they are found to be elongated. This condition depends upon the degree of distention of the vessels. Generally the epithelial layer presents on the ventricular surface an even, uninterrupted surface, so closely and evenly are the cells arranged. Tufts are present, however, where slight indentations projecting basalward between the cells are seen. Thus the rounded free ends of the cells, in cross sections, give the epithelial layer a corrugated appearance. Frequently cells are seen with bipartate free ends.

In the ordinary fixations and stains the cytoplasm in the majority of the epithelial cells stains evenly and compactly throughout. A very finely granular substance is distributed throughout this cytoplasm. No intracellular net work is seen. Vacuoles are frequently present. The nuclei vary somewhat in form. As a rule, they are spherical. In the more or less flattened epithelium they are oval in outline, with their long axes parallel to the surface of the tufts. Much chromatin is present in the nucleus although it does not stain solidly throughout, as is the case in many gland cells. Several large chromatin masses, as a rule, are seen in each nucleus surrounded by numerous finer granules. One or two nucleoli may be seen. These, however, are difficult to differentiate in the ordinary stains from the

larger chromatin masses. The nuclei occupy the central portion of the cells, perhaps somewhat more basalward than towards the free surface. Examination of many choroid plexuses does not reveal any marked variation to this position. I did not observe a nucleus compressed against the basal end of the cell, as is seen in many gland cells.

For a more detailed study of the surface epithelium, choroid tissues were fixed in Bensley's alcohol bichloride bichromate solution and formalin bichromate solution. Sections were stained with iron haematoxylin, copper chromehaematoxylin, Bensley's ('11) neutral gentian and safranin acid violet. In all these stains the epithelium stands out prominently. The cell membrane on the surface is seen as a relatively thick line, at times it appears as a double contoured line. No striations are seen in this cell membrane. After some fixations, where there is evidence that the process has not been perfect, the cell membrane appears as a thick margin, still retaining the original shape of the cell while the cytoplasm is shrunken away from it. In such preparations, the impression is given that these cells have thick, more or less unyielding membranes. No cement substance is present between the cells. Secretion granules, such as are seen in the pancreas, parotid, lachrymal, gastric and other glands, are not seen in the epithelial cells of the choroid plexus. However, after staining with neutral gentian or safranin acid violet, one finds cells which possess a few deeply stained granules that simulate in structure and staining characteristics the secretion granules of the above named gland cells. Such cells are not numerous, and the granules are only sparsely present, not more than one-half dozen have been counted in each cell. The nature of these granules has not been determined. They may represent nuclear substance within the cytoplasm. Occasionally one observes as well, large bodies near the nucleus which take the nuclear stain deeply. These may be interpreted as paranuclei and have been observed in various cells.

The epithelial cells possess numerous vacuoles and canaliculi. Others have observed vacuoles in the epithelial cells of the choroid plexus. Meek found fat globules in these cells in the rabbit,

which in prepared sections, owing to the dissolving action of the various preparation fluids, appeared as vacuoles. He proved that these were due to the former presence of fat, by staining with Sudan III choroid cells which had not been subjected to the dissolving action of alcohol and xylol. Globules, or vacuoles, have been observed in this epithelium by Luschka, Findlay, Studnicka, and Galeotti, which, according to these observers, are evidences of the vesicular type of secretion. On the other hand, Pettit and Girard are prone to regard the vacuoles as abnormal structures due to mechanical injuries or post mortem changes.

Numerous intracellular spaces were seen in my preparations notwithstanding that the greatest care was exercised to avoid mechanical injury to the cells. The tissues were removed and fixed almost instantly after the animal had been slaughtered, so that no postmortem changes could have occurred. These spaces are in the form of globules or vacuoles and canaliculi. The vacuoles may be seen in any part of the cell, either at the base or the free margin. They may be round or oval. Occasionally a row of these oval vacuoles are seen at the base of the cell.

The intracellular canalicular apparatus of Holmgren ('02), Bensley ('10) and others, may be seen in the cytoplasm as branching canals which frequently can be traced to the nucleus and partially encompassing it. They are entirely intracellular, as the branches terminate within the cytoplasm (fig. 3*a*).

That the finer vacuoles are cross sections of these canals is probable. Whether they bear any relation to the secretory activity of the gland, I am unable to say at the present time. That the larger vacuoles do not represent spaces remaining from dissolved fat globules is certain, for I have been unable to demonstrate the presence of such fat globules in the epithelium of the choroid plexus of the ox (figs. 3, *b*, *c*).

The description so far given for the epithelial cells is confined to those which are generally observed. Studies of numerous choroid plexuses, however, show various types of cells. In addition to those already described, one sees in fixed preparations

cells whose cytoplasmic area is composed almost entirely of vacuoles. The cytoplasm is manifested only between the vacuoles, forming a network surrounding the spaces. In another type, the entire cell is seen to be enlarged. It may be two or three times as large as the general type. It bulges out and is rounded as a consequence of its contents (fig. 2). This type shows that a confluence of vacuoles has taken place. This cell stains faintly because of the relative decrease in the cytoplasm.

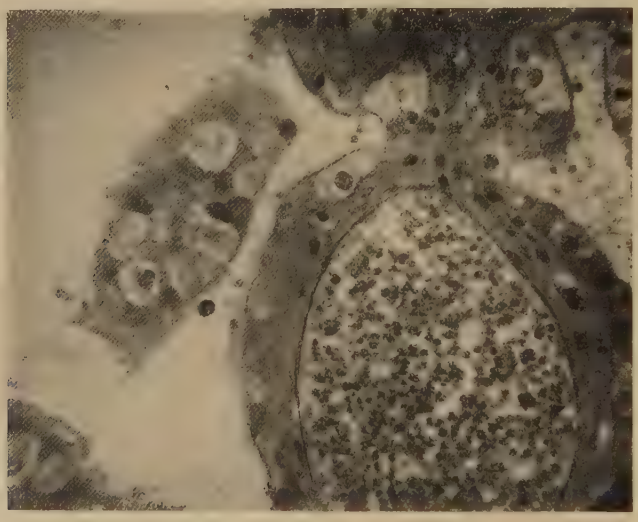

Fig. 2 Choroid plexus of the ox. Two types of epithelial cells are seen in the section: large, faintly staining, distended cells; and small deeply staining cells, which is the type usually making up the epithelium. Microphotograph, oc. 10, obj. 4 mm., B. & L. Tech.: Same as 1.

Only at the base of the cell is seen a narrow ragged zone of compact cytoplasm. In these large vacuolated cells, no changes are observed, as a rule, in the form and position of the nuclei, which are generally centrally located; however, I have observed the nuclei in the apices of many of them. The large bulging vacuolated cells may be seen between two smaller cells whose cytoplasm is compact. Again they may constitute the majority of cells on a particular tuft or villus.

That these various cell types belong to the same category and that they represent different secretion stages of the same cell type is evident.

From the study of these cells in fixed sections, one is constrained to believe that the secretion of the epithelial cells of the choroid plexus is a vesicular one. The secretion substance makes its appearance at the base of the cell as minute vesicles. Later they are seen throughout the cytoplasm. A confluence of these vesicles takes place to form large vesicles. At the same time the cell enlarges, becomes rounded and bulges out between the resting cells. Then the secretion passes out into the ventricles without a break in the continuity of the cell membrane.

Fig. 3 Three cells, selected, showing canals and vacuoles within the cytoplasm. *a*, intracellular canaliculi; *b* and *c*, vacuoles. Drawing somewhat diagrammatic, oc. 10, obj. 1.9 mm., oil immersion, B. & L. Tech.: Bensley's alcohol bichloride bichromate solution, neutral gentian.

That a continuous secretion is taking place is apparent from the fact that all the various secretory stages of these cells may be seen in one choroid plexus. One finds, however, some tissues in which the vacuolated cells are much more prominently present than they are in others. As a rule, the epithelium is of the general non-vacuolated cuboidal type which is described first.

The processes of secretion in the epithelial cells are in no way comparable to those of the duct gland cells,—salivary, pancreas, lachrymal glands. Secretion granules, the antecedents of these granules, and nuclear changes as seen in other gland cells are not demonstrable in the epithelium of the choroid plexus. The secretion substance of the latter makes its appearance as minute droplets within the cytoplasm and no antecedent secretion substances have been observed. A somewhat similar

method of secretion has been observed in the kidney by Gurwitsch and others. In other respects the secretion methods of the two organs,—choroid and kidney—are similar. In both large amounts of fluid are secreted (excreted) with but relatively little changes in the cells concerned in the secretion. The latter differs from the former in that no foreign substances, such as injected indigocarmin, potassium ferrocyanide, iron-ammonium citrate, prussian blue, have been observed to pass through the choroid epithelium into the ventricles. Experiments were performed on dogs by Prof. S. A. Matthews and myself in order to find some substance that, when injected into the blood, could be detected microchemically in the epithelium of the choroid. Our results will be published later.

The action of the choroid cells may be compared also, to that of the endothelial cells of blood vessels in the formation of lymph, providing we accept the theory that physical processes alone—filtration, diffusion and osmosis, do not explain all the phenomenon of lymph secretion, but that the endothelial cells are concerned in the secretion of lymph, in which case a large amount of fluid is produced without any distinct observable histological changes on the part of these endothelial cells engaged in lymph secretion.

Meek found that with an increase of cerebrospinal fluid following muscarin injection, the epithelial cells had increased in height and certain clear spaces formed in the apical ends of the cells. His observations corroborated in a way the observations of Pettit and Girard ('02).

I have not confirmed, in the opossum, the observations of Meek in this respect, but my observations are limited to a few adult animals. One must presume that the choroid plexus has an autonomic innervation like the salivary glands, etc., in order to secure this phenomenon. I have not demonstrated to my own satisfaction, either by the silver method or by vital methylene blue staining, that the cells of the choroid plexus are innervated.

I am inclined to hold that the choroid plexus cells and their activity in the secretion of cerebro-spinal fluid belong to the

category of endothelial cells and cannot be compared to duct gland cells derived from epithelium.

Definite intercellular spaces are also observed. These may be in the form of mere indentations between the surface ends of the cells or a definite cleavage may be seen reaching to the basal end of the cell, where it frequently appears continuous with other canal-like structures which enter the deeper connective tissue walls (fig. 4). Occasionally the intercellular spaces are globular in outline. These observations do not agree with those of Meek and others, who hold that the epithelial cells are so closely appressed that intercellular spaces do not normally occur and that

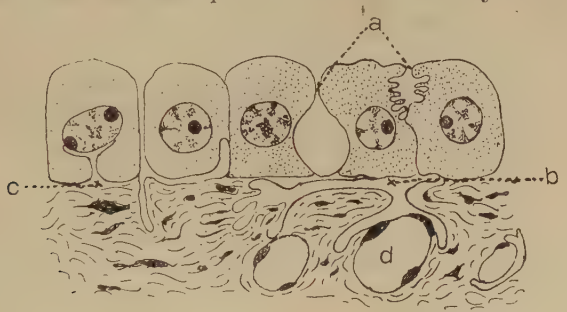

Fig. 4 Composite drawing of cells showing: *a*, intercellular canals; *b* and *c*, sub-cellular and interstitial canals; *d*, blood vessel. Drawing somewhat diagrammatic, oc. 10, obj. 1.9 mm., oil immersion, B. & L. Tech.: Same as 3.

when they are seen, they are due to faulty technique. Studnicka ('00), on the other hand, holds that these spaces are seen in the choroid plexus of the shark.

While I have not observed these intercellular and sub-cellular canals in living tissue, I am not convinced that they are always to be regarded as artifacts. The frequency with which they are seen, the variations observed in their outlines, point to the fact that they are normally present. The canals are frequently seen to continue as such in the connective tissue walls and apparently communicate with lymphatics. To maintain that they are normally present does not seem inconsistent when one compares the choroid plexus with other serous endothelial cells. In my opinion, these spaces can be readily compared to the stomata

described by v. Reklinghausen, Klein, Dogiel, and others between the endothelial cells of the peritoneum and by Ludwig and Dybkowsky in the pleura.

The silver precipitation methods were utilized in the study of these intercellular canals. A heavy black precipitation filling the entire epithelial cell occurs when the tissue is fixed in Kopsch's fluid. Thus when viewed through the low power of the microscope, this deposit in all the surface cells forms a black border around the choroid tufts. Projecting from the basal margin of this black border (the epithelial cells) into the connective tissue are seen numerous canal-like deposits which suggest the intercellular and subcellular canals. However, further observation is essential before I can come to any definite conclusion in this particular. The very heavy deposit of silver within the cells makes the observations difficult and one is not always sure as to whether he is dealing with definite tissue structures or with artefacts.

Further investigation is essential before one can state just what the function of these intercellular canals is. When compared again with the generally accepted function of peritoneal stomata, it is suggested that these canals may be absorptive in character, that is, the cerebro-spinal fluid may pass back into the circulation through them. If such is true, the choroid plexus then may be regarded as both a secretory organ and an absorption organ. On the other hand, the presence of the canals suggests another possibility, and that is, that they may function directly in the production of cerebro-spinal fluid. Should we accept the mechanical theory of secretion, it would not be fantastical to suppose that much of the fluid may be regarded as a transudate passing directly from the connective tissue surrounding the lymphatics and blood capillaries through these small sub- and inter-cellular canals directly into the ventricles, without passing through the epithelial cells. According to this hypothesis, the epithelial cells of the choroid plexus alone may not be concerned in the production of cerebro-spinal fluid. In view of the fact that large amounts of cerebro-spinal fluid may be secreted in a short time (200 to 300 cc. and possibly more in 24

hours in the human, Cushing '14), one might naturally expect to see more histological evidence of secretory activity on the part of the epithelial cells, providing these cells are solely concerned in the production of the fluid. Another observation that suggests this second hypothesis is that in the choroid plexus of the ox, numerous mast cells apparently pass directly from the blood vessels through the connective tissue wall and between the epithelial cells into the ventricles.

Against this view that the cerebro-spinal fluid may be regarded in part as a transudate is the fact that it differs from lymph,

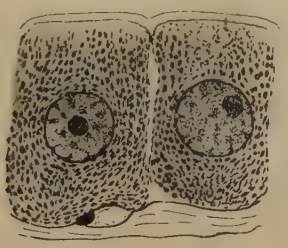

Fig. 5 Mitochondria in the epithelial cells. Drawing, oc. 10, obj. 1.9 mm., oil immersion, projection apparatus. Tech.: Bensley's acetic osmic bichromate solution, anilin fuchsin methyl green.

plasma, tissue juices and serous cavity fluids in the relative amounts of protein substance. Cerebro-spinal fluid is almost free from protein, while the other fluids contain much more.

That the choroid plexus is responsible for the formation of cerebro-spinal fluid was suggested by Faivre a long time ago. Luschka, a little later, agreed with him, and practically all observers since, including Pettit and Girard, Studnicka, Galeotti ('97), Carazzani, Meek, Mott ('10), Goldmann ('13) and Cushing, regard this structure as fundamental in the secretion of cerebro-spinal fluid. Cushing saw drops of the fluid exuding from this tissue. Goldman noted the extrusion of glycogen in the form of globules from the cells. Weed, in connection with others, suggests a dual origin of cerebro-spinal fluid—from the choroid plexus and from the perivascular systems of the nervous tissue.

Certainly much more work must still be done in order to solve the method of secretion which takes place in the choroid plexus.

When the tissue is prepared by Bensley's acetic osmic bichromate, anilin fuchsin, Methyl green method, the epithelial cells show numerous mitochondria. These are in the form of very short bacillus-like rods and tend in a way to arrange themselves in irregular rows, reaching from the base of the cell to the summit. Of course these rows are by no means so definite and clean cut as I ('16) have observed in the duct cells of the lachrymal glands (fig. 5). In the vacuolated cells, the mitochondria are seen in the cytoplasmic reticulum between the vacuoles.

INTERSTITIAL GRANULAR CELLS

This type of cell is characterized by the presence of numerous large granules which completely fill the cell. The nucleus is as a rule completely obscured by these granules which simulate in form and staining characteristics the secretion granules of gland cells.

These granular interstitial cells were found to be present without exception in the choroid plexus of the ox, but varied in number with different animals. In some, they were especially numerous, while in others only a few were found. Between these two extremes there was every gradation. In the sheep and swine they are not so numerous. In the choroid plexus of other animals examined—rabbit, guinea pig, dog, and human—these cells have not been observed.

The following description of the interstitial granule cells will be confined to those observed in the choroid plexus of the ox. The cells vary in size and shape. The average size is 20 micra. The majority are more or less spherical or oval in outline. Many, however, are angular, others are elongated or irregular and possess processes extending from the cells. They are situated in the connective tissue walls of the vascular tufts between the vessels' lumina and the surface epithelium. In some fields (low power microscope), as many as 30 of these cells have been counted (figs. 6, 7, and 8).

The thickest connective tissue walls, those surrounding the arteries, contain the largest number of these cells, which are found at varying distances from the lumina of the vessels. Some are seen in close contact with the endothelial cells of the vessels and in some instances, processes of the granular intersti-

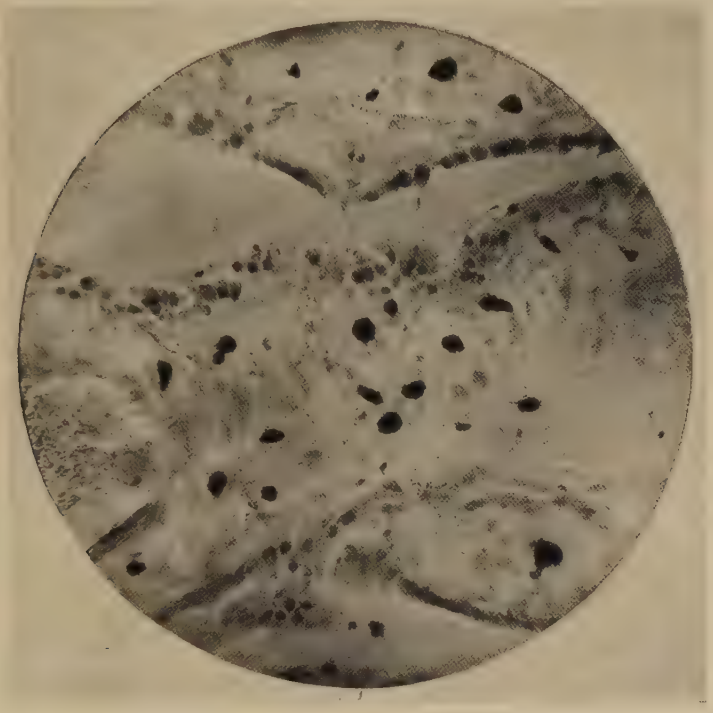

Fig. 6 Stroma of choroid plexus of ox. The numerous interstitial granular cells—mast cells—are deeply stained. Microphotograph, oc. 10, obj. 4 mm. B. & L. Tech.: Same as 3.

tial cells are seen to project between two endothelial cells into the lumen. The other extreme is seen where processes of these cells lying directly under the epithelial layer of the choroid plexus project upward between two epithelial cells into the ventricle. Between these two extremes the granule cells are seen in the connective tissue at different levels. None of these cells have been observed lying free either within the vessel lumina or on the ventricular surface of the choroid plexus.

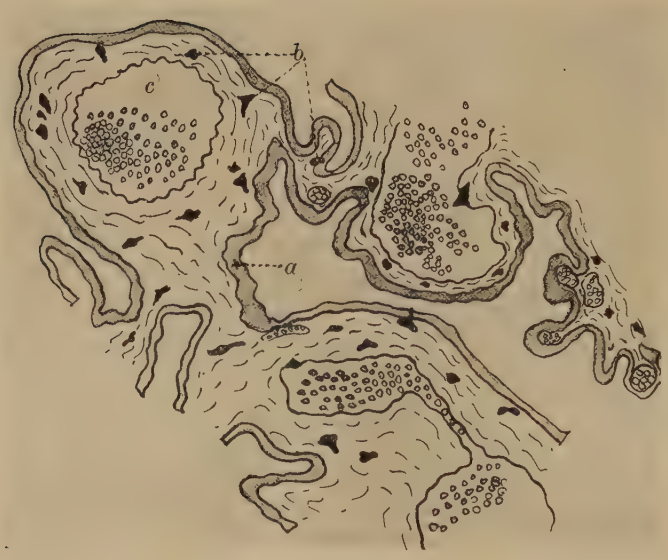

Fig. 7 Interstitial granular cells—mast cells—in the connective tissue wall. *a*, Surface epithelium; *b*, The deeply stained interstitial granular cells—mast cells—are seen at varying distances from the lumina of vessels. Some are directly underneath the epithelium with processes projecting between the epithelial cells. Others are seen directly underneath the endothelium of vessels; *c*, artery. Drawing, oc. 10, obj. 16 mm., B. & L. projection apparatus. Tech.: Bensley's alcohol bichloride bichromate solution, Unna's polychrome methylene blue.

Fig. 8 Four selected interstitial granular cells—mast cells—showing variation in form. Drawing, oc. 10, obj. 16 mm., B. & L. projection apparatus. Tech.: Same as 7.

The cells are readily seen in both fresh tissue mounted in serum or salt solution and in fixed preparations. Bensley's solution—equal parts of (a) saturated solution of mercuric chloride in 95 per cent alcohol and (b) 2.5 per cent aqueous solution of potassium bichromate—proved to be the most satisfactory fixing solution for the granular cells. They are fairly well fixed in alcohol. Fluids containing much acid destroy the granules, consequently Zenker's solution cannot be used. In Bensley's acetic osmic bichromate solution, the granules are only partially conserved. This fact, however, proves of great value because then the other constituents of the cell can be made out,—nucleus and intergranular substance.

The stains first utilized for the study of these cells were Bensley's neutral gentian and neutral safranin. The granules in these dyes stain deeply and hold on to the stain with much tenacity. Even after the section as a whole has been differentiated in alcohol-clove oil to the extent that practically all the stain has been removed, the granule cells remain deeply stained. And so numerous are these granules within the cell, as a rule, that the entire cell appears as one deeply stained mass. It is only after extended differentiation or in the processes of the elongated cells that the individual granules can be made out.

In these stains the nuclei in most cells are completely obscured by the deeply stained granules. Only in those cells where the knife has passed through the nucleus or in the smaller cells where only few granules are seen around the nucleus is the latter seen. It occupies a central position. It is oval in outline and vesicular,—thus staining faintly. Only a small amount of chromatin is present which is distributed throughout the nucleus appearing as fine irregular clumps. No nucleolus is observed. When these nuclei are seen through the low power of the microscope they appear as transparent or faintly opaque areas in the center of the cells surrounded by the deeply stained granules (fig. 9, *a*).

The presence of so many of these large round or oval mononuclear cells possessing numerous granules which simulate, both in size and staining characteristics, secretion granules suggested

at first the possibility of the choroid plexus containing some type of endocrinous gland (fig. 6).

Extracts of the choroid plexus were made in the usual manner and injected intravenously into a dog. Outside of the usual depressor reaction,—a frequent phenomenon accompanying injections of animal tissues,—no specific or particular reaction was observed. Dixon and Halliburton ('13) on the other hand claim that choroid extract when injected intravenously increase markedly the flow of cerebro-spinal fluid. Our observations, how-

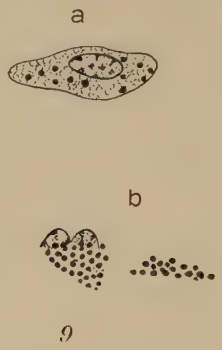

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Fig. 9 *a*, Interstitial granular cell, in which the large basophilic granules are not conserved. The mono-nuclear character of the cell is clearly seen. Note the fine intergranular network of cytoplasm. *b*, Broken mast cells, the granules of which are fuchsinophilic. Drawings, oc. 10, obj. 1.9 mm., oil immersion, B. & L. Tech.: Same as 5.

ever, were confined only to one experiment and consequently will not warrant any conclusion on this phase of the subject.

The probability that these interstitial granular cells are mast cells early suggested itself, especially after other stains had been used. However, I have not seen them so constantly and prominently present in any other tissue. Their presence in such large numbers may suggest that they have some function other than that generally ascribed to mast cells. However, our knowledge of the nature and function of mast cells is very superficial. Numerous theories have been advanced regarding the rôle they play in animal life. Many hold that they are in a way unicellular glands concerned in some type of secretion. If such be the

case, one may still regard these interstitial granular cells of the choroid plexus as having some unknown secretory function. At any rate, it was later determined that they possess the same morphological and staining characteristics as the so-called 'histiogenen Mastzellen' of Ehrlich and others. In view of these facts, the interstitial granular cells of the choroid may be regarded, for the present, at least, as belonging to this category, although their particular function here is unknown.

Since Waldeyer ('75) first described a particular group of connective tissue cells possessing granules, to which the term 'plasma cells' was applied, and later Ehrlich ('77) and his assistant Westphal ('80) recognized a still more limited group whose large granules showed a strong affinity for basic dyes and to which the term 'mast cells' was applied, much interest has been shown in these interstitial granular cells.

In the literature on this subject, mast cells are generally described as interstitial cells more or less round in contour, the cytoplasm of which is made up of large granules which have a marked affinity for basic stains, Mallory ('14), Schäfer ('12), Rauber Kopsch ('06). Most authors fail to differentiate between the two types first recognized by Ehrlich and Westphal, who referred to them as 'mastleucocyten' and 'Histogenen mastzellen.' Some writers describe the former and some the latter, when a definition of mast cells is made.

Regarding the two types, Prenant, Bouin, Maillard ('04) state: "Les cellules à granulations basophiles du sang sont les mêmes que les cellules nutritives que nous retrouverons dans les tissu conjonctif sous le nom de Mastzellen ou cellules-engrais." The two cell types were recognized by Pappenheim ('01) who discusses the origin of each, and by Maximow ('06) who states that the relation of the two types is not clear.

The distribution of mast cells, according to many investigators, is wide. This is true not only of the distribution in the individual, but also throughout the various orders of animal life. They have been described both in invertebrates, such as the cellules mucoides, described by Guenot in 'Gastropodes Pulmones' and in every order of vertebrates. It was the opin-

ion of early investigators that mast cells were found in the latter only in batrachians, where in the Urodeles they were seen in enormous numbers. Now it is generally conceded that they are found in all vertebrates. They have been described in triton, frog, turtle, rabbit, calf, man, and in fact, all species of vertebrates.

In the individual body they are distributed as follows: In the blood-basophilic leucocytes; connective tissue mast cells have been described in the connective tissue layers of the skin and mucous membrane. They are numerous in the tongue, in the septa of various glands, in the lungs. Arnold ('14) found that mast cells are greatly increased in numbers in the frog's tongue after induced passive congestion. Müncheimer ('95) saw them in the testes of horse, rat and pig. He failed to note them in these organs of the deer, sheep, dog and rabbit. Korybutt Daskiewicz ('78) describes them along the nerve fibers in the frog. McKibben ('14) states that they may readily be mistaken for nerve cells as observed in the nasal region and meninges of necturus. They are found in bone marrow, adipose tissue, along blood vessels, in newly formed connective tissue. These mast cells make their appearance early in embryonic life. They have been observed in the 9-day chick and in early calf embryos. These cells are much more clearly seen, as a rule, in the embryo than in later life.

I have failed to observe in the literature on this subject reference to these large, prominent interstitial granular cells—mast cells—in the choroid plexus of the larger mammals. Haeckel ('59) refers to certain interstitial cells in the choroid plexus of embryonal mouse and dog, which, according to him are similar to those seen by Schultze ('56) in the gelatinous connective tissue of tunicates and medusae. Haeckel considers these wandering cells and, according to him, they are filled with minute globules of fat.

I have stained the choroid plexus of the ox with a view of determining the presence of fat in the mast cells. Sudan III, Scharlach Rot—in alcohol or as Herxheimer's stain, and the Nile blues were used. So far, I have failed to demonstrate the

constant or prominent presence of fat in these mast cells. Only rarely are fat droplets seen. Hence the mast cells in the choroid plexus of the ox differ from those cells described by Haeckel. They are also different from the other mast cells of the connective tissue type in which fat and lipoid substance have been found, as claimed by Pappenheim, Posner, Lombardo and others. Huguenin ('12) saw numerous lipoid granules in mast cells in a case of status lymphaticus. According to Ciaccio ('13), lipoid and fat are found in these cells only under pathological conditions. Flemming ('71, '76, '79) and Hammar ('95) were of the opinion that fat was not deposited in mast cells. Certainly fat is not demonstrable as a constant constituent of the mast cells of the choroid plexus.

Reaction to mucin stains. One of the first stains used in the study of these cells was muchaematein, in which the granules stained a deep blue. No other elements in the tissue were stained. The presence of so many round or oval cells specifically stained in muchaematein or mucicarmin was another factor that suggested a glandular secretion phase to these interstitial cells. Of course, the presence of mucin naturally suggested itself. I ('16) have shown, however, that secretion granules—lachrymal gland—do stain specifically in these mucin stains, although we have no evidence that mucin is secreted by the lachrymal gland. Others have observed that the granules in mast cells are stained with mucous stain. Raudnitz ('83) held that because of this reaction, the granules represent a stage in mucinous degeneration of the cell. Hoyer ('90) held that the granules were of a mucinous nature. Schaffer concludes that as the mast cells take the same stain as do cartilage cells, the former contain a chondroitin sulphuric acid substance. On the other hand, Pappenheim, Schwenter, Trachsler and others held that the granules of mast cells have no relation to mucin. Ehrlich observed that the granules of mast cells are much more resistant to water than are mucous granules.

An interesting phenomenon in the study of muchaematein stained mast cells is the variability in the intensity of the staining reaction of the granules. Some cells take the stain much

more deeply than do others. One frequently secures choroid plexus in which the cells as a whole stain much more quickly and deeply than do the similar cells in other choroid tissue. This variety in intensity of staining with muchaematein may represent various stages in the formation of granules. No other elements in the cell are stained. Preparations according to this method are of great value in the study of the individual granules. These are seen to fill the entire cell and where processes are present, the granules extend into them to the very ends of the processes. They vary in size.

Reaction to basic stains. When stained in methylene blue, toludin blue, methyl green, etc., the granules are deeply stained. They hold these stains with such tenacity that when the sections are differentiated in alcohol, practically all the stain may be removed from the section before there is any perceptable loss of stain from the granules. The nucleus, when seen at all, occupies a central position in the cell and appears as an opaque or semitranslucent structure.

Iron haematoxylin and copper haematoxylin stains. The granules do not retain these stains after differentiation. In fact, the mast cells are among the first of the structures to give up the stain during the process of differentiation. The granules of the mast cells differ in this respect from secretion granules of many serous cells. The latter retain both stains deeply after practically all the iron and copper reactions have been removed from the section as a result of the differentiation.

Polychrome methylene blue stain. Here the granules stain metacromatically—a deep violet, while the remainder of the choroid tissue in general is only very faintly stained. The epithelial layer is tinged green. Here, as observed in other stains, the granules in the mast cells are so densely stained that the entire cell appears as a dark violet mass simulating an artefact. Prolonged differentiation in alcohol reveals the characteristics of the individual granules, especially in those cells where the granules are not so numerous. In many cells a translucent central area is observed which marks the position of the unstained nucleus.

In this fixation (Bensley's) and stain, the nucleus is never definitely seen because of the numerous granules that obscure it.

Alcohol fixation. The granules are not so well conserved in 70 per cent alcohol, although according to many observers, alcohol is one of the best fixatives for mast cells. This method of fixation, however, has its value, as the nuclei of the cells are plainly seen, owing to the fact that the granules are not well preserved. The nucleus is stained green (Polychrome methylene blue). It is very vesicular and is as a rule oval in outline. No nucleolus is present, as was also observed by Dantchakoff ('98), who states that no nucleolus is present in the nucleus of mast cells. Variable sized chromatin granules are present. As a rule one to four or five large chromatin masses, one to two micra in diameter, are seen in each cell. Interspersed among these chromatin granules is the fine chromatin dust. The chromatin is only sparsely present, which accounts for the vesicular appearance of the nucleus. The granules of the mast cells are almost totally destroyed when fixed in Bensley's acetic osmic bichromate solution. Here the nucleus can be readily observed. I was unable to demonstrate the presence of mitochondria in these affected cells after fixing in the above solution and staining with anilin acid fuchsin, methyl green. Occasionally, however, mast cell granules were seen which, instead of being stained green by the methyl green, were stained deeply red by the fuchsin (fig. 9, *b*).

When the tissue is prepared according to the method utilized by Cowdry ('16) and Scott ('16) for study of mitochondria, the granules of the mast cells are well preserved and are stained deeply green in contrast to the red stained mitochondria so abundantly present in the surface epithelium. I did not observe mitochondria within the mast cells. Had any of these minute fuchsinophilic granules been present, they should have been observed readily in contrast to the deep green stained mast cell granules. This observation is corroborated later on in the vital stains.

VITAL STAINING

Choroid tissue was obtained by gently separating the plexus from its attachment in the ventricle immediately after the animal was slaughtered. Small bits of the choroid were then placed in the following solutions: Neutral red, one part in 15,000 of isotonic salt solution; new methylene blue, one part in 10,000 of isotonic salt solution; janus green, Metz, 1 to 15,000; polychrome methylene blue, Unna, 1 to 15,000; and pyronin, 1 to 1,000. Small pieces were removed from each of these solutions, mounted in the respective solutions and studied under the microscope. The remaining tissues were fixed in ammonium molybdate solution. The following observations were made:

Neutral red stain. The choroid plexus is rapidly stained with this dye. Macroscopically, the entire tissue is stained deeply red. Microscopic examination reveals the following characteristics: The surface epithelium is stained deeply red, the stain is confined to the cytoplasm and is diffuse throughout. The numerous, minute, highly refractive granules which were observed in fresh, unstained tissue, stand out prominently in the diffusely red stained cytoplasm. The nucleus which is unstained is spherical and occupies a central position in the cell.

Numerous mast cells are seen, the granules of which stain rapidly and deeply red. The large oval nuclei of the mast cells remain unaffected by the stain. As a rule, they are much more readily seen in this preparation than in the permanent preparations. Contrary to the observations of Arnold, I noted some activity on the part of these mast cells when mounted in serum and enclosed within a stage incubator. One cell was seen to completely divide within the period of one and one-half hours. This particular cell, which was oval in outline at first, was seen to elongate itself by processes extending from both ends (fig. 10). The formation of new granules in the processes was concurrent with the development of the processes. With the elongation of the cell, a constriction occurred in its central portion, which continued until the cell was completely divided, and with the division of the cell, nuclei appeared in both halves. Of course it was

impossible to observe in this stain the method of nuclear division. Arnold saw no changes in the form and position of mast cells within a period of from twenty-four to thirty-six hours. Kanthack and Hardy held that the 'Histiogenen' mast cells are stationary. While Lowenthal, Maximow, Pappenheim and Weidenrich ascribed to them an amoeboid movement. Maximow saw alterations in the form of mast cells in inflammatory tissue.

In numerous other cells, processes were observed to extend out from the cell bodies and to work themselves between the con-

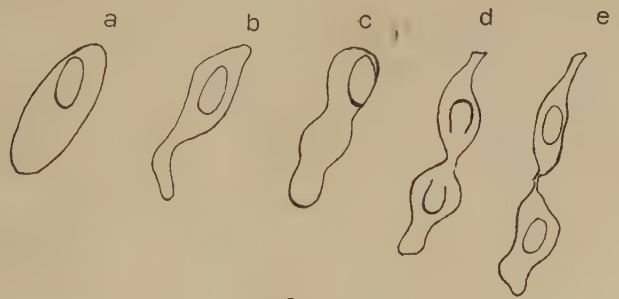

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Fig. 10 Outline of changes observed in a living interstitial granule cell—mast cell—during division. The time required was one hour and fifty-five minutes. *a*, 4.35 p.m.; *b*, 5.05 p.m., *c*, 5.50 p.m., *d*, 6.10 p.m., *e*, 6.30 p.m. Drawing, oc. 1–, obj. 1.9 mm., oil immersion, B. & L. Tech.: Fresh choroid tissue mounted in serum, vital staining with neutral red, electric incubator for microscopic stage, temperature, 98.5°.

nective tissue elements. Some of the processes developed within one-half hour. The development of the processes was characterized, as a rule, by a continuous out-flowing of granules from the cell body. Granules were seen, however, to develop in the processes some distance from other granules, thus apparently having their origin directly from homogeneous cytoplasm. In attempting to determine the origin of these granules, I have come to no conclusion. In many instances the granules appeared to divide. However, as noted, granules seemed to make their appearance directly from homogeneous cytoplasm. Many observers hold that the granules are derived from the

nucleus. Downey states that the chromatic substance comes from the nucleus, through its wall, and then comes in contact with the primary granules of the cytoplasm, after which the basic and metachromatic staining of the granules follow. My observations show that the granules may form in the processes some distance from the nucleus. Just what relation they bear to the nucleus is impossible to state. The fact that they take the nuclear stain is responsible for the assertions that they have a nuclear origin.

Polychrome methylene blue. Here the epithelial cells are characterized, when viewed from the surface, by relatively thick unstained cell boundaries which mark the polyhedral cell boundaries. The unstained spherical nuclei occupy the central portion of the cells. These are surrounded by unstained cytoplasm with highly unstained refractile granules. The nuclei, however, begin to stain early in this dye, thus indicating the toxic effect of the stain.

The granules of the mast cells rapidly stain metachromatically and the nuclei, as in the case of neutral red, are unstained. Later they begin to stain, as in the case of the epithelial cells.

Janus green. After the tissue has been in this solution for a few moments the connective tissue elements are stained, under macroscopic observation, metachromatically a pinkish violet or purple, while the epithelial cells are stained blue. When the tissue is studied by means of the oil emersion, it is seen that the mitochrondria of the epithelial cells are deeply stained blue, while the other elements of the cell are unstained. The mitochondria are numerous and show the same characteristics in both structure and distribution as observed in fixed preparations. A narrow, definite zone of homogeneous, unstained cytoplasm immediately surrounds the nucleus, in which no mitochondria are seen. This frequently gives to the nucleus a double contour appearance.

The mitochondria in the epithelial cells of the choroid plexus are indeed numerous. They are found in such great numbers that under low power the cytoplasm appears to be a solid blue throughout. It is only under the oil immersion that the minute

individual granules are observed. These granules vary in form. Some are spherical, others are slightly elongated, either slender or thick, others again are comma shaped. In no instance are long rods seen. It is the mitochondrial granules that are seen as the minute, highly refractive granules in fresh tissue, as well as in the various other vital stains used, where they remain unstained. In the janus green solution all the granular elements of the epithelial cells are stained blue. It is my opinion that the granules that have been described by previous investigators as secretion granules are in reality mitochrondria.

The mast cells are unaffected in this dye. Only rarely does one see granules stained with janus green. Frequently one observers a fine, intergranular deposit of blue stain between the granules of the mast cells. I was unable, however, to make out definite mitochrondria in this deposit.

Neutral red and Janus green. When both neutral red and janus green, mixed, are applied to fresh choroid tissue a very interesting picture results. The epithelial cells are deeply stained blue owing to the numerous mitochondria they possess. On the other hand, the mast cells are stained deeply red as a consequence of the affinity between the granules and neutral red. Careful examination of numerous mast cells did not reveal the presence of blue stained intergranular mitochondria. In fact, the complete absence of the latter was the striking and surprising feature, in view of the claims of Arnold and Dubreul that mitochondria are found in mast cells. Tochaschin ('12) reports staining of true mitochondrial elements in fibroblasts and clasmatocytes by isamine blue and trypan blue. In this preparation, when enclosed within a warming stage, I observed processes projecting themselves outwards from cells in which granules concurrently appeared but no mitochondria were observed. This observation is against the view that some have held –namely, that the granules of mast cells have their origin from mitochondria. These observations confirm those already described for the fixed preparations.

Methylene blue. Two preparations of this stain were used. Methylene blue rect. Grüber's, and Methylene blue, Metz.

The former stained both the epithelial cells and the granules of the mast cells a deep blue. In the latter stain the granules of the mast cells are stained metachromatically a reddish violet. In neither preparation were nerve cells and fibers observed accompanying the blood vessels. However, claims have been made that numerous non-medullated nerves are seen in the vascular walls of the choroid plexus.

Pyronin. The epithelial cells are stained deeply red. The stain is diffuse and confined to the cytoplasm in which the highly refractile, unstained mitochondrial granules are observed. The mast cells are not stained. No diffuse stain was seen surrounding the mast cells. This observation was made with a view of determining if a secretion substance is present which had been emitted from mast cells as a consequence of solution of the granules. Arnold obtained a red stained substance surrounding mast cells after vital staining with weak solution of methylene blue. This he interprets as a secretion, thus ascribing to mast cells a glandular function. Others have claimed that the granules of mast cells, especially leucocytes, go into solution. I was not able to verify this observation of Arnold, either in the pyronin or methylene blue stains.

NATURE AND ORIGIN OF MAST CELLS

Many theories have been advanced respecting the histiogenesis of mast cells. Lymphocytes, mononuclear leucocytes, basophilic myleocytes and bone marrow (Schridde, '96) are generally cited as giving origin to mast-leucocytes. That mast-leucocytes have a common origin along with other polymorphonuclear leucocytes is probable.

No unanimity of opinion exists, however, regarding connective tissue mast cells ('histiogenen mast-zellen'). That they have origin from mast-leucocytes and consequently from the same sources as the latter is held by many observers. Prenant, Bouin and Maillard regard the two as belonging to the same category. Ranvier ('00) held that his clasmocytes were mast cells, that they had origin from blood cells and that they were associated with inflammatory changes. Schreiber and Neuman ('01)

agreed with Ranvier that clasmocytes and mast cells belong to the same type. Maximow states that the relation of the two—mast-leucocytes and histiogenen mastzellen—is not clear. Ehrlich held that the connective tissue mast cell is a transformed or supernourished connective tissue cell, that the granules of the latter are stored albuminous substance, and that the cells are found where tissue juices are present in abundance. Baumer ('96) agreed with this conception. That the cells held a reserve substance was also claimed by Schneider ('02). Ballowitz ('96) found them to be especially numerous in hibernating animals. Others again hold that these mast cells are related to the formation of fat, as they are frequently seen in adipose tissue.

Unna ('91) saw the transformation of certain spindle or ellipsoidal cells into mast cells in healing syphilitic ulcers. Mast cells are frequently found under pathological conditions, such as in low-grade inflammations, urticaria pigmentosa, erythema multiforme, pleuritic exudates, etc. Joachim ('06) and Spilling described mast cell leukemias.

Some have made the claim that mast cells are not normally present in human blood. Michaelis ('02), Wolff ('02) and many others have found them to be normally present. Audry ('96) claims that connective tissue cells, large mononuclear cells, wandering cells and plasma cells may become mast cells. That these cells may have their origin from blood cells and vascular anlage was held by Marchand ('97).

Recently much attention has been given to discussions of the origin of vascular endothelium and blood cells. That the former may develop directly from the mesenchyme is claimed by McClure ('16), Reagan, Clarke ('16), Danchakoff ('16) and others. Clarke ('12) on the other hand maintains that lymphatic endothelium is formed from preëxisting lymphatic endothelium and that the mesenchyme retains throughout its identity as a mesenchymal cell. That red blood cells develop directly from endothelium (even when discontinuous and independent of the circulation, Reagan) has been shown by the works of Reagan ('16), Danchakoff ('16), Emmill ('16) and Jordan ('16). These observations are more or less at variance with the Angioblast

theory of His. Stockard ('15), on the other hand, did not observe in the embryo of the Teleost, Fundulus heteroclitus, any indications "that an endothelial cell has the power to produce a blood cell or to change into a blood cell of any type but much has been seen to the contrary." According to him, the "four distinctly different products differentiating from the apparently similar wandering mesenchymal cells" occur under the same environmental condition. His explanation of this phenomenon is that the original mesenchymal cells that wandered out were of four potentially different classes. "The four resulting types of cells are then in an embryological sense derived from different mesenchymal anlagen," although these cannot be differentiated. The term polyphyletic theory has been applied to this conception. Against this theory is the so-called monophyletic theory which is ably defended by Danchakoff and Reagan. In substance it is this: There is one common anlage for all blood cells, the later differentiation into the various types of blood cells is determined by environmental conditions.

To review the arguments favoring each theory would be irrelevant in this discussion. Danchakoff, after extensive observations on both the embryo and adult chicken concludes that all blood cells including mast cells, plasma cells and wandering cells have origin from a common anlage,—mesenchymal cells—and that this loose mesenchyme of a chick embryo (6–10 days) is equivalent in all the regions of localization and is polyvalent in its potencies of development. Further, Danchakoff found in the lymphatic nodes scattered in the loose connective tissue of the adult hen a loose syncytium which is considered as young undifferentiated tissue. From these loose syncytial cells amoeboid cells develop and these are the stem cells, or mother cells, of the various formed blood elements. Regarding the rôle that the stem cells play in the production of various blood cells and wandering cells, Danchakoff ('16) states:

> The erythrocytes, the small lymphocytes, the different leucocytes, the wandering cells of the connective tissue, the mast cells and the plasma cells—all these cells are different cell units, morphologically as well as physiologically. But in the early embryonic stages they all had

a common mother cell, and this mother cell is preserved in the adult organism and becomes the source of differentiation and regeneration and most probably also the source of pathological proliferation.

Many diverse opinions have been held regarding the nature of the granules of mast cells. Some have regarded them as albuminous in nature, while some regard them as mucinous. That they are associated with pathological changes in the cell and are products of inflammation or degeneration has been maintained by others. Stoffer held that they were related to the production of melanotic pigment. Pappenheim did not regard these granules as living,—'biophoren Plasmosomen'—but a 'substanz depot.' He bases this view upon his observation that the granules can be extruded from the cell without loosing their staining characteristics. Arnold, however, strongly maintains that mast cells are not to be regarded as cells in various stages of degeneration, but that they are active living normal cells. According to him, the presence of glycogen, fat, lipoids, pigment and mitochondria is sufficient evidence of this. Others hold that the mast cell granules are secretory in nature and consequently the cell is to be regarded as a unicellular gland. The chemical structure of the granules is unknown. According to many observers, they have their origin from the nucleus,—Weidenrich ('11), Downey ('13) and others. Arnold holds that they are related to mitochondria. Other substances found in mast cells, according to various investigators, are glycogen, fat, and pigments.

Many of the theories respecting the function of mast cells have been suggested in the two preceding paragraphs. In addition to these, Fahr has held that they exert a bactericidal or antitoxic action. That the cells are to be regarded as unicellular glands is an interesting conception. This view was held by Lavdowsky, Colleya and others, who claim that the granules become disintegrated, go into solution, and pass out of the cell to become mixed with the tissue juices, thereby contributing to the nourishment of the latter.

DISCUSSION AND CONCLUSIONS

The chief purpose of this paper is to call attention to the numerous interstitial granule cells in the choroid plexus of the ox, which possess the morphological and staining characteristics of mast cells of the 'histiogenen' or connective tissue type. Owing to the unavailableness of the ox, or the other mammals in which these cells were found in fewer numbers, for experimental purposes, there are many problems respecting these cells which remain unsolved for the present. One is almost constrained to believe that their origin is from the blood, for in sections they are seen first beneath the endothelial layer of the arterioles with processes extending into the lumina; then in the walls of the blood vessels at varying distances from the lumina, and finally beneath the epithelial layer of the choroid plexus with processes projecting between the epithelial cells into the ventricles. The entire picture suggests that the cells are trave ing from the blood vessels to the ventricles. The fact that they are seen in such variable numbers in different choroid plexuses strongly suggests this view. However, I have repeatedly attempted to find these cells in both the blood and cerebro-spinal fluid without success. It was my custom to draw off with a glass tube and rubber bulb from the ventricles, cerebro-spinal fluid whenever the opportunity presented itself. About 5 cc. of fluid can be obtained in this way from the ventricles. Of course, there is some admixture of blood with this fluid, owing to the customary methods of slaughtering these animals. The fluid was then centrifugalized.

I have been unable to confirm that these granular cells regularly pass from blood vessels to the ventricles, although permanent preparations strongly suggest such an activity. Further, I have not observed mast cells passing from blood vessels in living tissue when mounted in serum.

My observations so far do not disprove the hypothesis of Ehrlich that they are highly or over-nourished connective tissue cells. It is feasible to assume that they are wandering cells, or a special type of connective tissue cell that have become granular as a consequence of their migration through the connective tissue

wall of the choroid plexus. That they may originate entirely within the connective tissue wall is suggested in view of the fact that they were seen to undergo cell division. Certainly there is a large amount of cell nourishing substance—cerebro-spinal fluid—passing along the course of these cells from the blood vessels to the ventricles.

Should we accept Ehrlich's conception, the mast cell cannot be regarded as a specific cell, but rather as a condition of a more or less general type of connective tissue cell. Against this theory, however, is the fact that the formation of granules occurs simultaneously with the development of processes and that in cell division the granules are abundantly seen in the two daughter cells during the process of division.

That they are not specifically related to fat formation in the choroid plexus seems certain, for fat is found in this tissue only in very minute quantities.

The cells are in no way related to any demonstrable pathological conditions. Hence the assumption that they represent abnormal processes is untenable. Likewise, they cannot be considered as related to the formation of pigment.

That the cells are concerned in some endocrinous secretion early suggested itself. Their presence in such constant and large numbers, their structure and staining characteristics, which in many respects resemble that of other gland cells, might indicate this. It was not until basic stains were used and the cells were seen to stain metachromatically with polychrome methylene blue that they were placed in the category of mast cells. Notwithstanding this fact, they may be concerned in some type of secretion. Many observers still regard mast cells as having a secretory function. Against this view of the secretory function of these cells may be advanced the fact that they are found only in a few mammals—in the ox, in great abundance, less numerously in the sheep, and only sparsely in swine. Further, no demonstrable physiological action was obtained after injection of ox choroid extract into the dog. I was unable to demonstrate a pericellular secretion substance, as observed by Arnold. Proof is still wanting that these cells are concerned in the secretion of some specific substance.

The apparent migration of these cells through the choroid plexus from the blood vessels to ventricles and the presence of inter- and sub-cellular canaliculi suggest that the cerebro-spinal fluid, at least in part, may follow such a course and consequently be independent, to some degree at least, of the surface epithelial cells.

The chief value of this paper is to point out that the choroid plexus of the ox offers excellent facilities for teaching and study of mast cells.

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INTESTINAL HERNIA IN TWO SPECIES OF FROGS

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THREE FIGURES

In considering the survival of the fittest we often find examples in man and other mammals of individuals who are somewhat handicapped by mental and physical defects but who are able to exist by their own labors. While intestinal hernia is not a great hindrance to normal activities in the case of civilized man, it is often a contributing cause to the death of some domesticated or wild mammal.

Recently two cases of hernia of the small intestine in the frog came to my notice and seemed worthy of mention in view of the paucity of recorded cases of such defects in vertebrates below mammals.

The first specimen, a young bullfrog (Rana catesbiana) was shown to me by a student in General Zoology, Mr. Charles Hruby. It was an apparently healthy and vigorous male with a body length of $3\frac{3}{4}$ inches. No anomaly was visible externally but the small intestine was curiously out of position. From within 4 cm. of the pylorus to within 3 cm. of the rectum the duodenum and ileum were entirely protruded through a small aperture in the abdominal wall to occupy a position in an enlarged lymph space just under the skin of the right side (figs. 1 and 2).[1] The pancreas was about 2 cm. long and lay in the mesentery between the pyloric end of the stomach and the duodenum. The duodenum extended about 2 cm. beyond the pancreas and became directed to the right side of the animal, finally passing through the muscular wall. Through the same opening, the ileum extended to join the rectum. Examination of the parts extruded showed them to be much coiled and closely

[1] Photograph by Mr. F. H. Dodge, drawings by Mr. Richard Ashman.

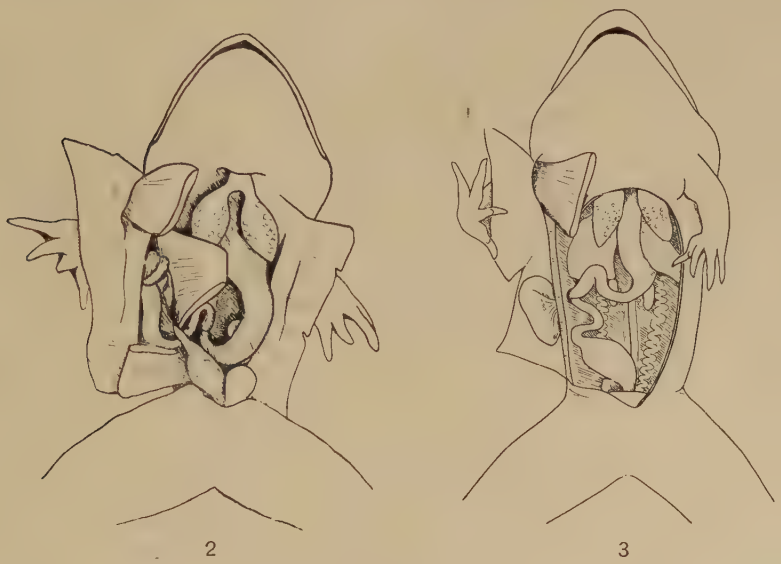
folded. The coiled portion was a little more than 8 cm. in length when straightened. The spleen was in its normal position in the abdominal cavity and the rectum and stomach were perfectly normal. There was no anomaly of the reproductive or

Fig. 1 Photograph of intestinal hernia in Rana catesbiana

excretory systems, and the blood supply of the whole intestine was complete.

The second specimen was presented to the writer by a former student, Mr. T. C. Nelson of the University of Wisconsin, who kindly offered to add it to my teratological collection.

It was an adult male leopard frog (Rana pipiens) measuring 3 inches body length and externally presenting no appearance of deformity. Careful examination of the organs other than the intestine showed them to be normal (fig. 3). The duodenum extended about 4 cm. from the stomach as in the larger specimen, then passed out through the body wall and continued as a single loop for 5 cm. before it recurved and passed through the same aperture into the body cavity and connected with the rectum at



2 3

Fig. 2 Intestinal hernia in Rana catesbiana
Fig. 3 Intestinal hernia in Rana pipiens

a distance of about 3.5 cm. In both cases the cavity was soft walled and showed but slight thickenings of its walls due to abrading action of the intestine.

It is possible that early in the tadpole stage when the first elongation of the duodenal loop occurred, there was enough force exerted to break the body wall opposite the curved intestine; this seems quite plausible when we note that the extrusion took place at about the same point in the two specimens. Another less likely possibility is that mechanical injuries (on the right side

in each case) caused by crayfish, fish, frog, or turtle, produced sufficiently large apertures to allow the growing intestine to force its way out. In any case it is evident that frogs may live apparently unhampered by quite considerable hernias of the small intestine.

OBSERVATIONS ON THE INFLUENCE OF ISOLATED OVARIES ON THE BODY GROWTH OF THE ALBINO RAT (MUS NORVEGICUS ALBINUS)

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TWO CHARTS

Previous experiments had shown (Stotsenburg '13) that the removal of both ovaries from a young rat was followed by an acceleration of body growth. When one ovary only was removed, the other being left untouched, the body growth was not modified (Stotsenburg '13) but the remaining ovary underwent a marked hypertrophy (Hatai '13).

The experiments to be reported were designed to test the question whether the exclusion of the reproductive function of the ovary, by isolating it from the uterus, would produce any general change in body growth.

Two series of experiments were made:—one series in which both ovaries were isolated and left in place—designated 'double isolation'—and a second, in which one ovary was isolated and left in place, while the other was completely removed—designated 'single isolation.'

TECHNIC

Each test animal underwent two operations—about ten days apart. In the case of the double isolation the procedure was as follows: About 1 cm. of the right uterine horn was removed at the first operation—and at the second, the same length of the left horn. In the single isolation the first operation was as given above—but the second consisted in the complete removal of the left ovary. All the operations were successful. The albino rats were thirty-two to thirty-five days old at the time

of the first operation and forty-five days old at the time of the second. At the date of the last operation and for the first forty-five days after the last operation, the body weights were taken at intervals of fifteen days, then at intervals of thirty days, until the termination of the experiment at two hundred days.

In the further description of the work the two series of experiments will be treated separately.

DOUBLE ISOLATION

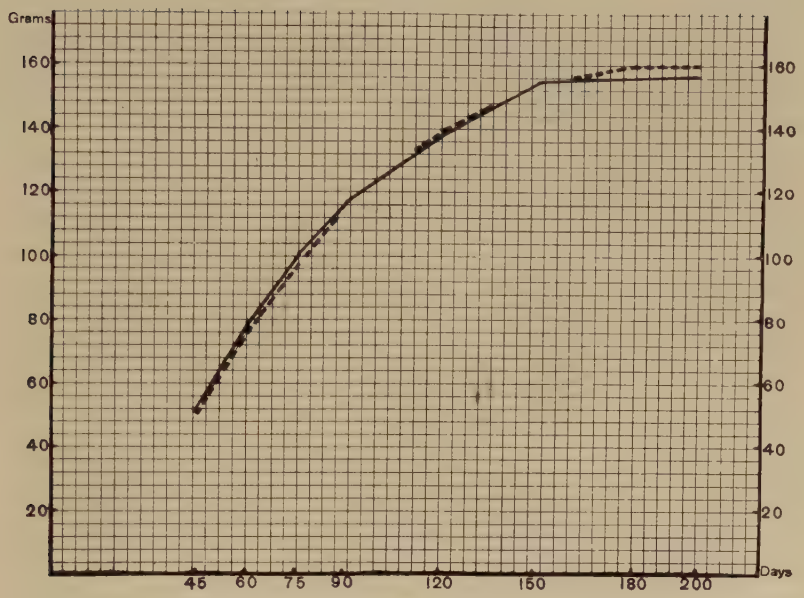
By the method just described, both ovaries were isolated in 20 females which were compared as to their body growth with 16 controls of like age. These 36 animals were from 7 litters, and as far as possible the same litter was made to furnish both control and test animals. The growth of the controls and of the test animals after double isolation is given in table 1 and shown in chart 1.

TABLE 1

Giving the average body weight of 20 test rats—'double isolation' and 16 controls, from which the graphs in chart 1 were plotted

AGE IN DAYS	TEST ANIMALS. NUMBER OF ANIMALS	DOUBLE ISOLATION AVERAGE BODY WEIGHTS	CONTROLS. AVERAGE BODY WEIGHTS	CONTROLS. NUMBER OF ANIMALS
		grams	*grams*	
45	20	49.4	51.1	16
60	20	76.5	79.2	16
75	20	98.5	101.1	16
90	20	116.0	116.7	16
120	20	140.0	138.0	16
150	20	154.0	150.0	16
180	20	160.0	156.0	16
200	20	160.4	156.8	16

From these data it is evident that this operation does not modify the growth of the body in weight. An inspection of the isolated ovaries at the time the test rats were killed yielded the following: There were 20 animals examined, in 7 rats both ovaries were not evidently diseased, but in 6 of these they were under weight and in one animal over weight. On the average these 14 ovaries were 13 per cent less in weight than was to be



expected from the tables for the normal ovaries (Donaldson '15). In 12 rats there were pathological changes. In 3 rats both ovaries were pathological. In 9 rats one ovary was pathological. In one case one ovary had disappeared. There were therefore in all 15 pathological ovaries to be considered. In the 12 rats with pathological ovaries—showing cysts—there were 15 cysts. These were distributed 11 on the left side and 4 on the right side. The first and earlier operation was on the right side.

SINGLE ISOLATION

For this series 17 test animals were subjected to the operation for 'single isolation' as previously described. There were 14 controls, and 7 litters were represented in the entire series. Test and control animals were taken as far as possible from the same litter. The growth in body weight of the rats after single isolation is given in table 2 and represented in chart 2.

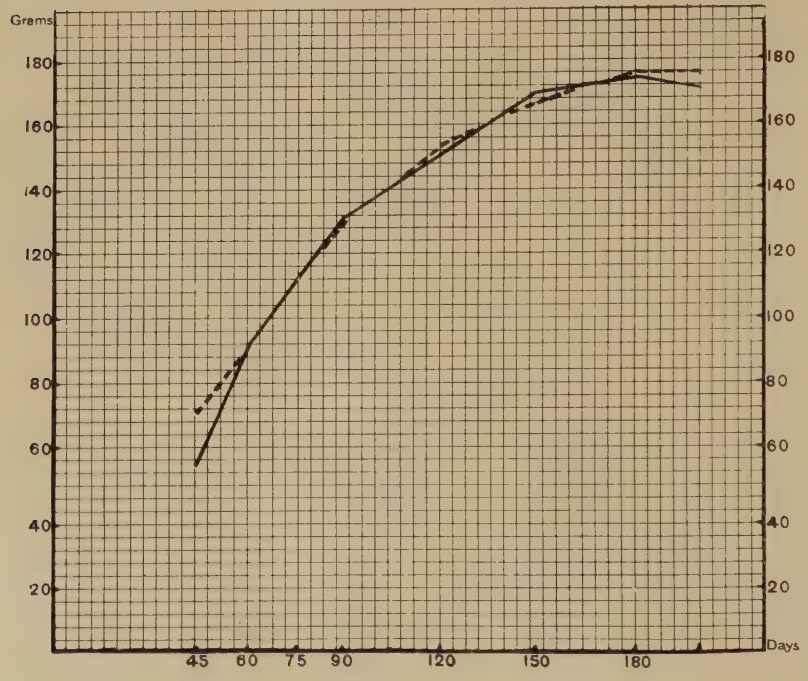
As shown by these data, isolation does not modify the body growth. An inspection of the isolated ovary at the time these rats were killed yielded the following:

TABLE 2

Giving the average body weights of 17 test rats—'single isolation'—and 14 controls, from which the graphs in chart 2 were plotted. Several animals in the test series were removed at one hundred and eighty days because of lung infection, and several after one hundred and fifty days from the control series for the same reason. Test animals—single isolation.

AGE IN DAYS	NUMBER OF ANIMALS	TEST ANIMALS. AVERAGE BODY WEIGHTS	CONTROLS. AVERAGE BODY WEIGHTS	CONTROLS NUMBER OF ANIMALS
		grams	*grams*	
45	17	69.6	56.9	14
60	17	90.0	90.7	14
75	17	112.3	113.9	14
90	17	130.3	131.4	14
120	17	144.6	150.9	14
150	17	166.9	171.0	13
180	14[1]	175.6	174.0	12
200	15	175.3	171.1	11

[1] Weighing of one rat accidentally omitted.



There were 15 test animals examined. In 10 the ovary was not diseased. In 5 the ovaries were pathological, being represented by cysts. The weights of the 10 normal ovaries were as follows: one was 44 per cent less than the table value, the remaining 9 were all above the table values. On the average these 9 remaining ovaries weighed 148 per cent more than normal. They were therefore considerably over twice the normal weight to be expected from the reference tables (Donadlson, '15). It is to be noted that in the reference tables the weights are given for both ovaries taken together.

CONCLUSIONS

1. Both the double and single isolation experiments show that the glandular function of the ovary which affects body growth is unmodified when the ovary is isolated from its connection with the uterus—since the isolated, acts like the normal ovary to inhibit growth.

2. This inhibition of growth is exercised in the case of 'double isolation' by ovaries that appear under-size or pathological at the end of the experiment (200 days), while in the case of the single isolation experiment—ovaries, for the most part greatly hypertrophied, exercise the same control.

3. From the fact that the same effect follows from ovaries in such different conditions it would appear that they probably exercise their control through the mediation of some other less modified member of the endocrine system.

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ANTERIOR HAEMATOPOESIS IN CHEMICALLY TREATED TELEOST EMBRYOS UNDER CONTINUAL OBSERVATION[1]

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ELEVEN FIGURES

Inhibition of the movement of body-fluids has recently been employed for the purpose of obtaining information concerning the normal origin of teleost blood corpuscles. Those investigators who have employed this method of study have emphasized the importance of a thorough knowledge of the history of the experimental material from which conclusions are to be drawn. For instance, it has been stated (10, p. 579) that if an investigator knows the entire history of a given embryo, having determined during that time the embryo in question has never had any circulation whatsoever, he will invariably find that blood cells are located only in the intermediate cell-mass and on the posterior and ventral yolk surfaces; that there may be some variation but none "sufficient in any case to confuse the problem;" that erythrocytes in such embryos (8) will never be found in the anterior mesenchyme, anterior vessels, anterior yolk surface, heart or liver, "in any embryo at any age;" that in these latter regions (9, p. 315) wandering blood anlagen never make themselves manifest. Along with these statements, no claim is made that their author has followed in sufficient detail the history of the development of any embryo to justify an assertion that the complete history of any embryo was known. Perhaps the most

[1] The present communication represents a portion of a work involving continual observation which was aided by a grant from the National Academy of Sciences.

definite available statement in this connection (10, p. 578) is the following:

"Since one is able to be *absolutely certain* that the blood never circulates in a great number of embryos, only such embryos should be considered in a study of blood origin."

It is evident that if one should use as his criterion for lack of circulation a corresponding lack of erythrocytes in certain locations—if he should refuse seriously to consider all embryos with erythrocytes in these locations because of pictures which can be obtained in embryos by the arrest of a once established circulation, he is using a method capable of giving only one result.

On the other hand, it has been suggested (5, p. 104) that this same thoroughness of knowledge of a given embryo's history, such as could be obtained only by constant observation, might conceivably heighten the probability of a detection of erythrocytes in those locations in which they have been claimed never to originate.

It has been stated that circulation, after having once been established, may be lost temporarily intermittently or permanently. Temporary or intermittent loss of circulation would, of course, lead to error of interpretation, provided that circulation were of a sufficiently elusive nature to take place only between those intervals at which a single investigator could reasonably be expected to make his observations. It seems that the only means of meeting this difficulty of elusive circulation (if there be such a phenomenon) is to examine as often as possible the experimental material. Since the working hours of a single investigator might be inadequate for the elimination of the objections which might be raised by believers in elusive or intermittent circulation, the problem would best be attacked by the coöperation of a sufficient number of observers that the probability of detection of an elusive circulation approaches a maximum. It is, however, impracticable to observe with absolute constancy any single embryo. In the first place, it is impossible to predict which chemically treated embryos of a large number will succumb to treatment, develop a circulation, or develop without a circulation. Since embryos of the latter sort are generally few

in number compared with the other two sorts, constant watching of only one individual would necessarily involve a waste of time, and would no doubt be superfluous, even if one could be sure that a given embryo would develop without a circulation. At any rate, the method in the present work was continually to eliminate from a group of chemically treated embryos, those individuals which developed a circulation, and those which died or became extremely abnormal. Such groups of treated embryos were carefully observed day and night at intervals varying from thirty minutes to an hour or slightly more. Low and high power binocular microscopes were employed in these observations. In order that individual cases might receive adequate attention, it was necessary to limit the number of embryos very considerably below that which might be studied if observations were to be made only a few times each day.

The primary purpose of the present work was to obtain a definite answer to the simple question: are erythrocytes ever found in the anterior mesenchyme or in any anterior vessels of teleost embryos in which the blood has never circulated? A negative answer to this question has been insisted upon by Stockard, while a positive answer has been likewise insisted upon by Reagan. The possibility of migration of erythrocytes or their anlagen is entirely beside the question. The issue concerns the presence or absence of erythrocytes in this location regardless of all possible means of attaining that location with the exception of passive movements induced by heart pulsation.

Reagan (5, p. 116) has pointed out the fact that Stockard (8) has described erythrocytes as 'originating' on the yolk when their ultimate anlagen must have come from another location; the former has also pointed out the fact that even the leucocytes and their anlagen, as described by Stockard, were unable to migrate. In consideration of ultimate origins it is well to remember that the intermediate cell-mass itself arises by the gross mesial displacement and subsequent union of two longitudinal columns of mesenchyme.

It may be stated at once that a small number of embryos which developed without circulation, as was determined by fre-

quent observation, possessed erythrocytes in their anterior mesenchyme. It has previously been admitted (5, p. 112) that a large number of embryos without circulation may fail to exhibit red blood cells in their anterior regions. It was first pointed out by Reagan that blood shares with other tissues the especial susceptibility of the anterior end of the embryo to a derangement of metabolism. One can readily obtain large numbers of embryos devoid both of circulation and of anterior erythrocytes; but if the middle and posterior regions of such embryos were found to be as greatly arrested and abnormal as the anterior portions generally are, they would be rejected as material in which to study the origin of the vascular tissue or the origin of any other tissue. This specific behavior of the anterior tissue is included among the extensive studies by Child on Axial Gradients. To confirm the work of Stockard, one must reject all embryos exhibiting anterior erythrocytes regardless of their histories, and maintain that in the remaining embryos with diminutive anterior ends, erythrocytes have developed in all those places in which they could ever develop in the normal individual; one must claim that there is no possibility of varying degrees of haematopoetic potentiality. It seems unreasonable to assume that all regions should exhibit equally strong tendencies to produce blood cells, or that they should all necessarily produce them at the same early period of ontogeny. Further study alone will determine the reasonableness of this assumption.

The material for the present work consisted of a few hundred embryos of Fundulus heteroclitus, which, at a very early stage, were treated with chemicals. The conditions in one of several satisfactory embryos obtained (i.e., having anterior erythrocytes) will suffice for descriptive purposes. The known history of this embryo will be given in detail. The eggs constituting the group from which this embryo was taken were fertilized at 4 p.m., June 22, 1916. At the two cell stage, they were placed in a solution consisting of 70 cc. of sea water to which had been added 10 cc. M/12 butyric acid. Four hours later the solution was diluted by the addition of 50 cc. sea water. In this mixture they remained until the total time of treatment amounted

to twenty-four hours, after which they were reared in sea-water which was frequently changed. From the time of their removal from the solution of butyric acid the eggs were frequently observed. Recorded observations were commenced on June 26 at 8 a.m., although at this time there was no sign of endothelium on the yolk-sac. Examinations were made as follows:

June 26, a.m., 9.20, 10.30, 11.45; p.m., 12.20, 1.30, 3.30, 4.45, 5.34, 6.11, 7.00, 8.05, 9.02, 10.00, 10.55, 11.54. June 27, a.m., 1.00, 2.32, 4.00, 5.15, 6.30, 7.50, 9.00, 10.15, 11.20; p.m., 12.10, 1.30, 2.11, 3.26, 4.19, 5.30, 6.14, 7.35, 9.00, 10.55. June 28, a.m., 12.30, 2.00. (So far there had developed no pigment on the yolks and none of the embryos possessed pericardia. Constant observation up to this time was quite superfluous.) 3.12, 4.15, 4.45, 6.00, 7.00, 8.02, 9.14, 10.25. p.m., 1.45, 3.14, 4.15, 5.11, 6.30, 8.02, 9.00, 9.55, 11.00. June 30, a.m., 12.20. At this time the embryo from which figures 1 to 11 are taken was found to contain anterior erythrocytes; this embryo was thoroughly studied, sketched and then preserved in picro-acetic acid. It might be of interest to note that none of the (approximately) one hundred individuals obtained in this same treatment had yet established a circulation. A little later, one of those embryos was found in which heart-pulsation was able to cause oscillation of the blood-corpuscles. This embryo was discarded; then twelve hours elapsed before another such embryo was found, observations having been kept as constantly as in the earlier stages.

One embryo in which anterior erythrocytes (figs. 1, 9 and 11) were observed, and the history of which has been given, is of great interest. The yolk-sac was observed just before the preservation of the embryo to be quite devoid of endothelium except in the most posterior region. This endothelium was readily found in section. Numerous pigment cells had developed on the yolk sac. Also there were many oil globules. On the posterior yolk were some large groups of developing blood cells which had yet shown little indication of possessing hemoglobin. They were detected by means of a high power binocular. Their color was only a little different from that of the surrounding mesenchyme, but their shape and refracting power were characteristic. In the posterior region of the intermediate cell-mass the developing erythrocytes had assumed a faint yellow color. There was not the slightest evidence that any of these cells had been, or were being, displaced by any sort of movement of body-fluids. No

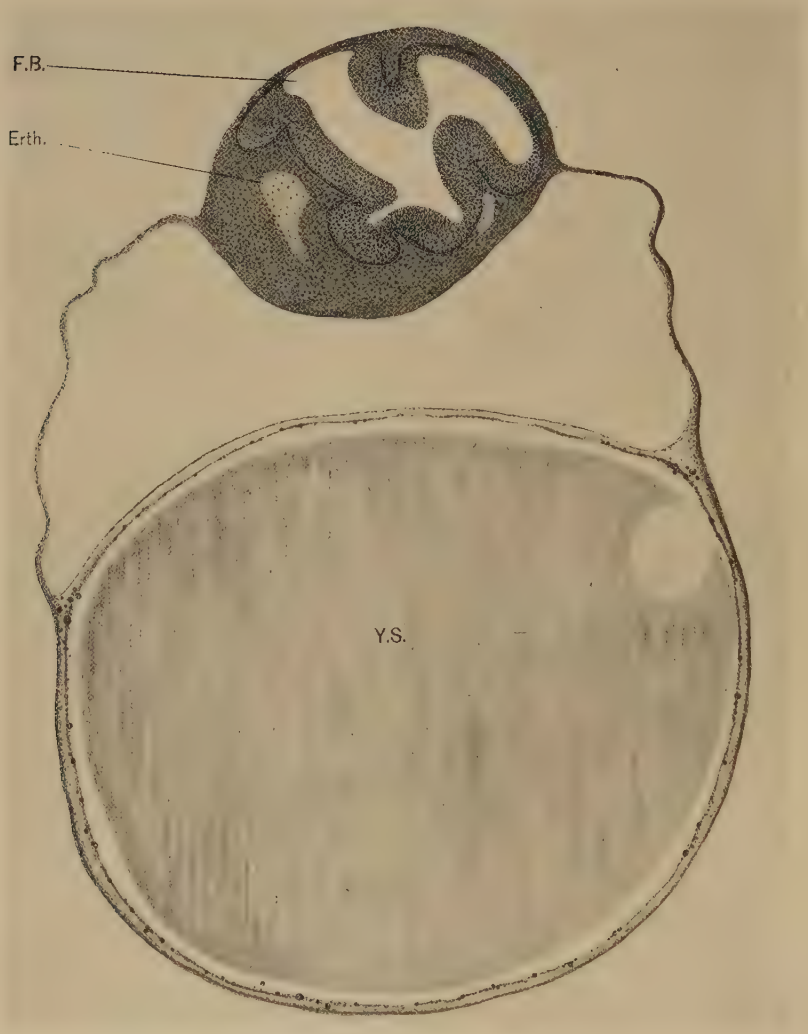


Fig. 1 Section through the anterior body axis and yolk sac of a chemically treated embryo from which all other figures in the present account were taken. The section shows lacunae of erythrocytes in the anterior mesenchyme. The yolk-sac is seen to be quite free from vascular tissues. Normally such a section should contain a cross-section of the heart. In this embryo the venous attachment of the heart is dorso posterior in the pericardial cavity. Continual observation exp. 2.53, 1916. *Erth.*, erythrocytes; *F. B.*, fore-brain; *Y. S.*, yolk-sac.

body-movements could be elicited from this embryo. The heart was very inconspicuous (fig. 8) and was detected with great difficulty. A very slight twitching could be observed on the ventral side of the head which gave much the appearance of slight irregular contraction of a body-muscle. The wave of contraction was directed anteriorly, appearing superficially to be quite opposite in direction from that generally found in embryos at this stage. This circumstance finds its explanation in the fact that the venous attachment of the heart to the yolk-sac is situated at the extreme dorso-posterior limit of the pericardium ventral to the axial body-tissue (figs. 8 and 10); in the normal individual the venous portion of the heart is attached to the yolk at the antero-ventral limit of the pericardial cavity. In this specimen there is no endothelial tissue near the venous end of the heart. Also there are no blood corpuscles near it, though two cells were found among the myocardial cells which had become faintly eosinophilous, and one was much rounded. The endocardium is represented by a very delicate cord of cells which are not sufficiently numerous to enclose a cavity (fig. 6). The myocardium is weakly developed. Considering the fact that this embryo is less than six days old, it seems improbable that any yolk sac endothelium should have formed and then degenerated. That endothelium which could be observed in the living condition was readily found in section. At any rate, continual observation established the fact that there was no circulation. In the living condition the body tissue was clear and of healthy appearance. It seems quite likely that the prevention of circulation in this instance may have been due to an inherent abnormality of the heart. The anterior end of this embryo was so little affected, judging at least from external appearances, that a circulation might well have developed later, provided the heart had attained its normal relationships. It is impossible to state to what extent the chemical treatment is responsible for this heart abnormality.

Embryos of this sort are very desirable for study. In many instances a local and apparently insignificant abnormality may be sufficient to prevent circulation in embryos whose general con-

ditions are such that a circulation might well be expected. Such embryos are certainly as useful, as far as the lack of circulation is concerned, as those in which the agent of arrest of circulation has produced generalized abnormality with specific njury to the anterior tissues. It is in the former type of embryos that anterior erythrocytes are most often encountered.

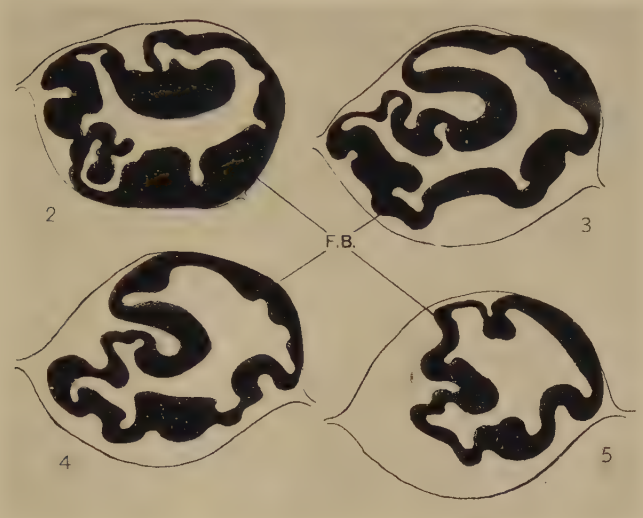
In figure 1 it will be seen that blood cells have developed in the anterior mesenchyme of the optic region, and the median dorsal fore-brain region. As has been stated, these cells possessed more hemoglobin than did the more posterior ones, though the former might easily have been overlooked in the living embryo.

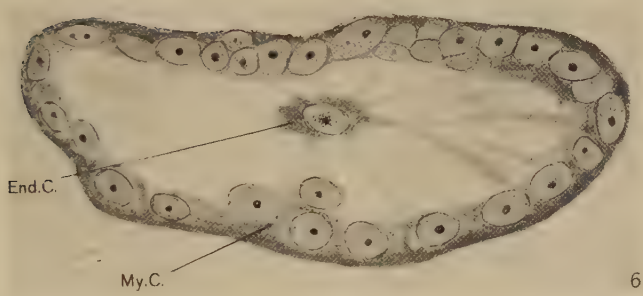
The fore-brain tissue in this embryo appeared in the living condition to fill an abnormally large part of the head-region. The head-region itself seemed to be perfectly normal in size, differing in this character from the great majority of embryos in which circulation was prevented. In the fore-brain region, rather unusual developmental procedure is in evidence. There are several evaginations from the brain which bear a resemblance to optic cups. On each side there is a large cup and some smaller ones. Both dorsally and ventrally the fore-brain has given off evaginations which in some cases are so fused with the body-wall ectoderm (ectoderm in case of the dorsal and lateral ones) that no line of demarcation can be detected between the two ectodermal tissues (fig. 2). This hyperactivity of the fore-brain tissue is sufficiently often accompanied by the excessive development of anterior erythrocytes to warrant the suggestion that the two phenomena may be causally connected. Reagan (5, p. 107) has described a case in which supernumerary prosencephalic evaginations were accompanied by anterior erythrocytes.

Figs. 2 to 5 Sections through the fore-brain, showing hyperactivity of that tissue (encephalocoels). Exp. 2.53, 1916.

Figs. 6 and 7 High-power drawings of sections through the heart of this same embryo. Figure 6 lies in the arterial region. The endocardium consists of a single strand of cells. *Endc.*, endocardium; *Myc.*, myocardium.

Fig. 7 Section through the venous end of the heart in which neither myocardium or endocardium is distinguishable from mesenchyme.


2
3
F.B.
4
5


End.C.
My.C.
6

7

In this connection the work of Child (2) is of extreme interest. He has found that long and severe treatment eliminates or prevents the formation of median or axial tissues, thus causing bilateral structures to fuse; that this fusion is most pronounced in the anterior-most tissues and progresses gradually backwards; that the originally distinct eye-spots of Planarians can thus be caused to fuse into a single median one. Werber (13) suggested vertebrate cyclopia to be an expression of this same sort of procedure. As a matter of fact the vertebrate otocysts themselves may be caused to fuse into a single median one which surrounds itself with a single otic capsule. As yet it had occurred to no one to suggest that the ears arise from a median anlage, as has been maintained for the eyes. But it has also been found by Child in a work which is not yet published, that while the anterior end suffers this specific injury with some treatments, other methods of treatment less severe and of slighter duration preceding removal to the normal environment will produce conditions under which the anterior end recovers with greater rapidity than does the posterior end, and a condition of hyperactivity may exist. Child was able by these latter methods to produce four-eyed Planarians. These results on Planarians are paralleled recently by the unpublished work of Child on Echinoderm plutei. By long and severe treatment, it was found that the morphologically anterior skeletal elements may be made to fuse, while in the case of weaker and shorter treatments, the angle of divergence between them may be made to approach 180°, while other anterior tissues become correspondingly hyperactive. While the recorded instances of anterior hyperactivity are few in the case of vertebrate embryos under treatment, it is possible that methods might be obtained whereby such hyperactivity might be obtained more often than by methods at present known to us. Quite independently of the results of Child, we have found as he has found in his unpublished work, that hyperactivity of the anterior end is often obtained when embryos are exposed to strong solutions for a very short time, followed by weaker solutions before removal to the normal environment. From such treatments, however, embryos may be obtained which appear

to have suffered neither arrest nor over stimulation in the anterior tissues. While such embryos may exhibit many anterior erythrocytes, the lacunae observed there are not so large as in those embryos whose anterior ends have been overstimulated.

There is one point of great importance in regard to the staining properties of early erythrocytes. When these are stained with methyl blue and eosin, or when they are stained with Wright's blood stain, the staining reaction is practically the opposite of that of older erythrocytes. Also the staining reaction of early erythrocytes in a single embryo may be opposite or very different in different regions.

In blood-smears from early embryos, such as four-day embryos, which seem to have been most successfully stained, the nuclei of the blood cells (all of which appear to be erythrocytes) stain reddish purple or red, while the cytoplasm appears blue or even densely blue and faintly granular; this is the picture obtained from Wright's blood stain. In eosin-methyl blue, the nucleus stains red or purple, while the cytoplasm appears dense blue, pale blue, greenish blue or in some cases very slightly purple. These staining-reactions of course may vary with the technique, but the general results above described are most often obtained.

In sixteen-day embryos, or even in stages younger than this, when the red corpuscles have well developed haemoglobin, the staining reaction is practically opposite that of erythrocytes of four-day embryos. In these advanced stages the best-stained preparations show erythrocytes with deep blue or purple nuclei and with deep red, pink or almost clear cytoplasm. If one employs such stains as the haematoxylins, the nuclei of all stages can be made to stain darkly so that even with a counterstain, the method seems incapable of demonstrating the nature of this remarkable change which takes place.[2]

[2] A reversal of staining reaction of nucleus and cytoplasm was observed many years ago by Auerbach. Wallin (Anat. Rec., vol. 9, no. 6) observed a reversal of staining reaction in mesenchyme cells and in free blood-cells in the *anterior* mesenchyme of the larva of Petromyzon. Also this result was obtained from ordinary stains. No doubt the entire chromatin content of a cell

A most interesting case of variation in the staining reactions of erythrocytes within a single embryo is that exhibited by the individual which is figured in the present communication. As has already been noted, the anterior erythrocytes were more deeply red in the living condition than were those of the posterior region, the latter appearing only faintly yellow. It was possible to mount enough of the sections on one slide so that the staining conditions may be assumed to be sufficiently uniform in different regions to warrant the belief that actual differences are portrayed. The nuclei of the anterior erythrocytes stained homogeneously red and purple while the cytoplasm stained pink. The erythrocytes in the posterior part of the intermediate cell-mass were found to possess red and purple nuclei of granular character and pale blue or densely granular blue cytoplasm. The intervening regions exhibited transitional conditions between these extremes.

These peculiarities of the staining reactions of erythrocytes are worthy of consideration in connection with the anterior 'leucocytes' described by Stockard (9, pp. 280 and 281). It is interesting to note that these 'leucocytes' were found almost entirely in embryos about seventy-two hours old. It may be regarded as highly significant that their first recorded counterpart in the normal (?) series is found in a sixteen-day embryo, although the statement is made (9, p. 236) that the entire experimental series was checked by corresponding normal stages. The mere fact that these particular cells "stained differently from any other cells within the embryo" or that they are 'peculiar' (9, p. 280) certainly comprises insufficient grounds for their interpretation as leucocytes. This hiatus in the production of leucocytes between the ages of three and sixteen days is most puzzling. If it be true, as suggested by Reagan (5, p. 113)

may become oxychromatic. It may, however, be doubted whether Wallin is really dealing with acidophylic chromosomes. He shows no distinct chromosomes. It is possible that in his figures 5 and 6 he has cut diagonally through a spindle so that at one pole the section passes through a sheath of oxychromatic substance while at the other it passes through the central core of basichromatic. The latter, however, should in all cases show at least a thin periphery of oxychromatin.

that these are really abortive attempts at erythrocyte formation, and that these cells have, or have had the potentiality of elaborating hemoglobin, it seems difficult to explain the fact that they were never detected as erythrocytes in stages slightly later than seventy-two hours unless these were then discarded on the assumption that the blood had circulated. Stockard (10, p. 578) regards all the 'beautiful blood islands' and 'great clots' in the anterior end of the embryo as mere 'pitfalls' to correct observation. But if embryos containing anterior lacunae of erythrocytes must necessarily have had a circulation, it should still be possible to find these 'leucocytes' somewhere in the embryo. Diligent search for such leucocytes in embryos of this sort has many times proved fruitless.

A careful study was made of some of these embryos which were able to develop circulation following the chemical treatment; this involved frequent observation of about twelve hundred embryos. In every instance in which a circulation was known to have existed and then to have stopped, the embryo failed to re-establish a circulation and soon died. Two hundred embryos, furthermore, which had established circulation subsequent to chemical treatment were placed in a solution of 0.01 per cent potassium cyanide to which was added a rather strong solution of acetone. Examined after having been subjected to this severe treatment for one and three-quarters, five and one half, eighteen and one half, and twenty-eight hours, the embryos were found without exception to have retained their circulation. At the end of forty-eight hours only two of the two hundred embryos had lost circulation; these quickly disintegrated without having resumed circulation.

From the foregoing results, the conclusion seems justified that the intermediate cell-mass and the posterior and ventral yolk surfaces are not the only locations in the teleost embryo which are capable of giving rise to red blood cells. Just to what extent any experimental condition represents the normal is difficult to say. The fact that a great many embryos with arrested development fail to show anterior erythrocytes might conceivably militate against the belief that the anterior mesenchyme normally

contains erythrocyte anlagen. It is, however, a very significant fact that the more normal the general conditions to be found in such embryos, the more likely they are to develop anterior erythrocytes. In the greater number of cases of developmental arrest, those conditions which will prevent circulation will also prevent the formation of anterior erythrocytes.

It seems probable that when an embryo is once able to establish a circulation, it has little tendency to lose that circulation under favorable environmental conditions. When loss of circulation does occur, the embryo usually dies. We have found no case of temporary loss and re-establishment of circulation—much less, any sort of elusive or intermittent circulation. Also there has been no instance in which a pericardium was observed to become oedematous or the heart string-like, once the circulation had been established. We do not assert that such things never happen. As a matter of fact, one does well to refrain from sweeping generalizations of a negative character in discourses dealing with the developmental possibilities of chemically treated embryos.

It is not our purpose to apply the facts above presented to considerations of mono- and polyphyleticism, except to state that they furnish a positive disproval of one of the contentions upon which support of the polyphyletic view has recently been based. There are certain facts which can be determined; beyond this, all is mere speculation. One can determine three things concerning the origin of a tissue: first, whether it arises from a narrowly limited anlage; second, whether any other tissue can ever give rise to the tissue in question; third, whether the assumed anlage never gives rise to any other tissue except the one for which it has been claimed to be specific. If the development of a given tissue fails to conform to any of these three possibilities, polyphyleticism can never be proved.

There seems to be no little confusion as to the proper application of the results of the study of vascular tissues in the teleost to the Angioblast theory. Stockard believes that the studies on yolk-sac endothelium by Wenckebach, Raffaele, and himself, help to disprove the Angioblast theory.

Sabin (Science, August 4, 1916, p. 155) states: "In his (Stockard's) studies made on the yolk-sac of the living fish embryo, he found that endothelium arises as spindle-shaped cells which differentiate out of the mesenchyme. Moreover, he found that the endothelial cell was distinct from the blood cell. This confirmation of the angioblast of His I regard as very important."

The truth is that the observation of local formation of endothelium on the fish yolk-sac or on any other yolk-sac proves or disproves neither of the conflicting views. Both views admit of locally formed yolk-sac endothelium. The fact that the endothelial and corpuscular end-products of differentiation are distinct lends no more support to the Angioblast theory than to the Local Origin theory. If they were not distinct, how could we diagnose them? The statement that two differentiated products are different is simultaneously so correct that it is redundant and so redundant that it is correct. Their distinctness does not preclude community of descent; this is a fact which we grasp with great difficulty. The real problem is whether we will recognize or refuse to recognize the transitional stages between the end-products, provided such can be found. Until recently, most of us have refused to recognize the transitional stages which undoubtedly exist between mesenchyme and endothelium, and now that we have done so, there is a movement to attach to individual cells in this transitional stage the appellation 'Angioblast,' so that whereas there was originally one angioblast there are now as many thousands of them as the case may require. It is true that certain words in the sciences have undergone evolution in meaning; this is true of the word 'cell,' but this evolution did not serve to confuse the issues of a controversy. Inasmuch as Hooke did not consider a cell to be a vacuum, the term is not so inappropriate as it is sometimes considered.

Stockard objected vigorously to the account of the development of the blood in Keibel and Mall's Human Embryology for the reason that here the monophyletic view is (3, p. 350) accepted 'with open arms' (9, p. 310). But this account is written in strict accord with the Angioblast theory. It is difficult to reconcile polyphyleticism and angioblast, if angioblast be a 'unit

anlage' (3, p. 499) of which all vascular tissues are 'direct descendants'.

There have appeared recent discussions over blood-histogenesis in which authors would seem to believe themselves to have settled by words the entire question of preformation—a feat which Bonnet, about two centuries ago proclaimed as "une des plus belles victoires que l'entendement put ait remporte sur les sens."

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PLATES

PLATE 1

EXPLANATION OF FIGURES

8 Sketch of the living embryo of which all other figures in this account represent sections or portions thereof. The erythrocytes in the head, tail and on the posterior yolk are represented by dots. The entire yolk sac except the posterior surface is devoid of blood and endothelium. There are large oil-globules on the yolk. Exp. 2.53, 1916.

9 Portion of a section through the fore-brain region in which the hyperactive brain-wall has fused with the ectoderm, enclosing on all sides some mesenchyme which became haematopoetic. There is no endothelium present. If any has been present it has either disappeared or turned into erythrocytes. *F. B.*, fore-brain; *Ect.*, ectoderm; *Erth.*, erythrocytes.

10 Section through the body-axis of the same embryo showing the loose mesenchyme (*mch*) in which the venous end of the heart loses itself.

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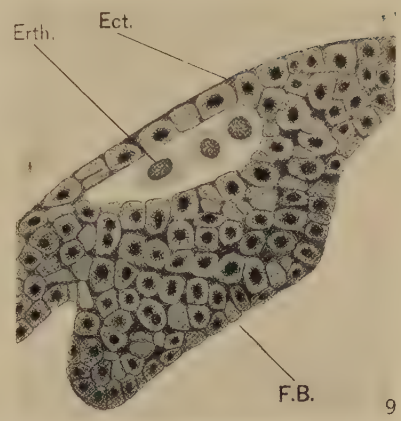

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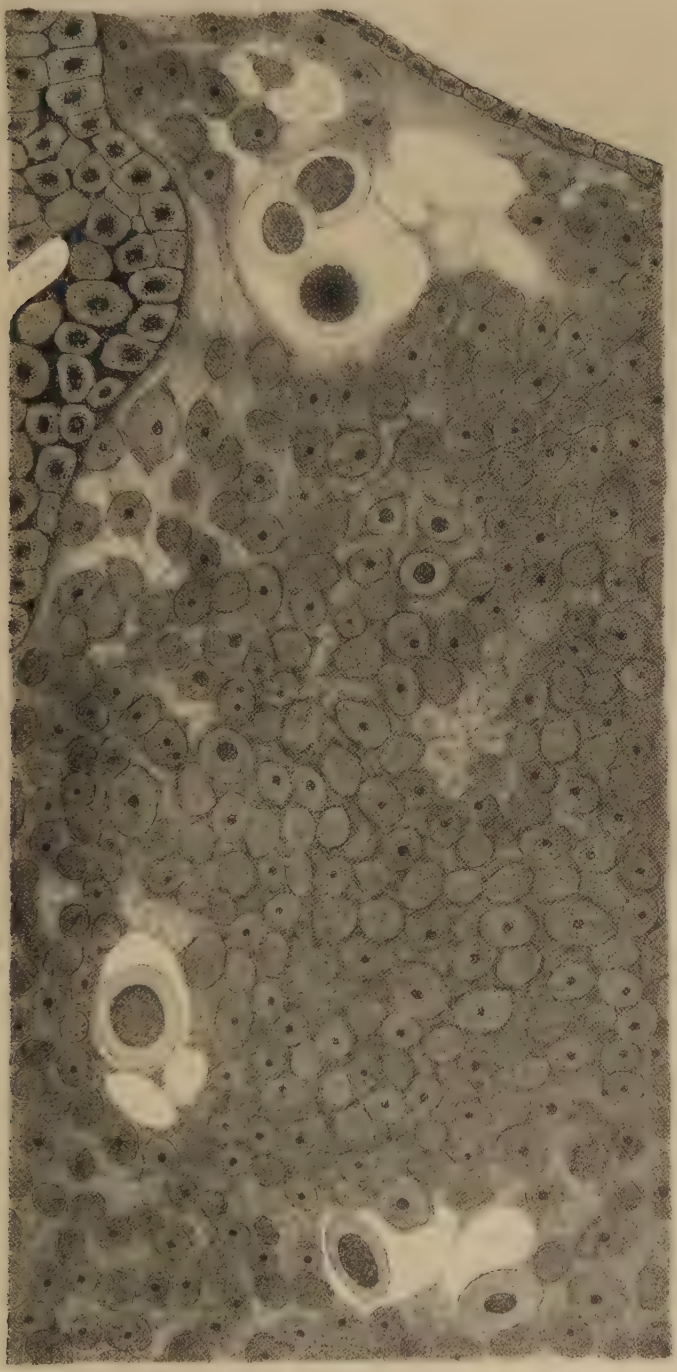
PLATE 2

EXPLANATION OF FIGURES

11 Portion of a section through the fore-brain region of the embryo from which the previous figures were made. It was drawn at high magnification. Lying free in the mesenchyme are erythrocytes apparently in various stages of development. The epithelium with large cells and dark intercellular substance is prosencephalic tissue.



OESTRUS AND OVULATION IN SWINE

GEORGE W. CORNER AND A. E. AMSBAUGH

From the Anatomical Laboratory of the University of California

I. THE PERIOD OF OVULATION

It seems remarkable that almost nothing should be known of the process of ovulation or of the mature ovum of the pig, which has for many years been in constant use for the investigation and teaching of mammalian embryology. In so thorough a review as Marshall's 'Physiology of Reproduction' ('10) there is only a meagre note:

> It is probable that the sow ovulates during oestrus and not during the pro-oestrum, since it is stated that sows are most successfully served on the second or third day of 'heat.' Coition, if it occurs earlier, is frequently not followed by conception. From Hausmann's description (1840) it would seem that ovulation does not take place prior to coition, but this statement has not been confirmed.

That ovulation occurs at or near the time of oestrus is stated in Keibel's Normentafeln ('97) and is of course implied in Assheton's studies on the early development of the pig ('98), but the precise relation of the two events has never been fully worked out.

To obtain exact information upon this point we have undertaken the observations here reported. They have been made possible through the generous cooperation of Mr. J. O. Snyder, of the Western Meat Company, San Francisco,[1] and of Mr. Ralston B. Brown, Superintendent of the Oakland Meat and Packing Company's plant in West Berkeley, and his staff. Through Mr. Brown's kindness permanent laboratory space had

[1] We hope to have an opportunity to express fuller thanks to Mr. Snyder and the gentlemen of the U. S. Inspection service at the South San Francisco plant, in connection with other studies to appear somewhat later, the material for which has been obtained through their immediate assistance.

already been provided at the packing house; for this work we were allowed free access to the stockyards at all hours, and upon occasion animals were killed out of the regular order for us.

The females of the wild swine of Europe are monoestrous, according to Kaeppeli ('08), having but one period of heat in the year; but under domestication the sow becomes polyoestrous, coming in heat at intervals of two to four weeks, usually about every twenty-one days, as all breeders agree. The period of heat commonly lasts three days and is characterized by sexual excitement and in some individuals by swelling, reddening, and slight eversion of the vulva, or even at times by a serous, mucous, or partially sanguineous discharge from the genital orifice. If a boar be present, the sexual excitement is made apparent by ready acceptance of coitus, which is denied at all other times; if none but females are in the pen, the sow in heat will be seen to sniff at the genitals of her neighbors and 'ride' them in imitation of coitus. Frequently the sow is the recipient, rather than the donor, of these attentions. The period is not terminated by coitus, but continues until the end of three days.

In order to distinguish these sows from others in the corrals until the time of butchering, they were marked with a daub of white paint thoroughly rubbed into the hair of the back between the shoulders. Immediately after evisceration, the Fallopian tubes were removed by cutting across the upper portion of the uterine horns, were carried to the laboratory in 0.7 per cent saline solution, and there washed out by inflating them with salt solution through a slit in the wall near the fimbriated extremity. After inflation with the fluid, the tubes were gently 'milked' into a Syracuse dish, and the washings examined with the dissecting microscope. This simple and almost infallible method of finding the ova was suggested to us by Professor Evans as an improvement on Martin Barry's practice of milking the tube without injected fluid ('39). As we have subsequently found it had been used by Sobotta and no doubt others as well.

Our series includes ten animals which were in heat on the day of killing or the day before. In eight of these the Graafian

follicles had ruptured and we were able to recover some or all of the ova from the tubes in each case. Of these eight sows, six were killed on the second or third day of the period, one between sixteen and thirty-nine hours after the onset of heat, and one between thirteen and twenty-two hours after the beginning of oestrus. In two of the ten there were large Graafian follicles, all unruptured except one follicle in one of the sows, which had apparently just collapsed. Unfortunately we have no record as to the time of onset of heat in these two animals, but the conditions in the other eight show that ovulation had occurred during oestrus, and probably on the first or second day of the period.

II. OVULATION SPONTANEOUS IN THE SOW

During the discussion which arose over Born's and Fraenkel's suggestion that the corpus luteum exercises the function of inducing ovulation at regular periods, a distinction was drawn between those mammals in which ovulation is spontaneous, and those in which copulation is necessary to invoke rupture of the follicles. Villemin ('08) maintained that ovulation is spontaneous in all mammals, but Ancel and Bouin ('09) state, on the basis of personal researches (details of which are not given) that ovulation is spontaneous in the human species and in other primates, in the dog, horse, cow, and pig; in the rabbit, guinea-pig, mouse, and cat rupture ensues only after coitus. The work of Marshall and Jolly ('06) on the dog and Heape ('97) on the mare, are in agreement with the results of Ancel and Bouin, and to the first mentioned class we may also add the sheep (Marshall '03) and the rat, according to Sobotta and Burckhard ('10) confirmed and extended by the recent carefully gathered data of Long and Quisno ('16). The placing of the rabbit in the second class has been confirmed by Regaud and Dubreuil ('08); the cat by Longley ('11); and Marshall ('04) has added the ferret to the list. The mouse, however, belongs to the class in which ovulation is spontaneous (Tafani '89, Sobotta '95) and also the guinea-pig (Loeb '11).

The only mention of the sow in this regard is the statement of Hausmann ('40) quoted above from Marshall ('10) that in this species ovulation is not spontaneous. On the contrary, our specimens show clearly that in swine coitus is not necessary for rupture of the Graafian follicles, for we have records of ten sows in which ova were found in the tubes although no boars had been in the pens with them. Mr. Brown informs us that according to the conditions of shipment of the live-stock, it is very unlikely that these animals had the opportunity to copulate before arriving at the stockyards. Two of the sows were under observation before oestrus set in, and are therefore even more definitely known not to have copulated. Moreover, in an animal in which but one follicle of many had ruptured, copulation had been observed sixteen hours previously. Ovulation, therefore, is independent of copulation.

III. THE MATURE OVUM

Little or nothing has been known of the mature ovum of the sow, and we have found no record of any previous observation of the unsegmented ova from the tube. Assheton's earliest specimens were already in the two-cell stage ('98). Lowrey ('11), in his study of the prenatal growth of the pig, attempted to estimate the size and weight of the mature ovum by allowing a slight addition to the diameter of the largest ovarian ovum he found, which measured 177 micra, with a zona pellucida 10 micra in thickness. He estimated, therefore, that the mature ovum would have a diameter of 180 micra. We have measured fourteen fresh tubal ova from nine sows, and find the diameter, including the zona pellucida, to vary from 155 to 165 micra, the zona being about 10 micra in thickness. The ova are plainly visible with the naked eye if placed against a strong light. We have not noticed a radial striation of the zona pellucida either in fresh or fixed ova. The ovum is filled with yolk granules of varying sizes, usually about three to five micra in diameter, which are so numerous and so refractile that they quite conceal the nucleus. A polar body may often be seen very clearly.

The ova are usually naked, but may be covered by the cells of the corona radiata, or even by a considerable portion of the discus proligerus.

A few of the ova which have been sectioned, seem to show no deviation from the usual process of maturation in other mammals; the first polar body and the second polar spindle are formed in the ovary, and the second polar body seems to be formed after fertilization. In each of two sows killed on what we believe to be respectively the sixth and eleventh days after the onset of oestrus, we were surprised to find a degenerating ovum in the tube.

In three sows in which copulation had occurred, fertilized ova were found. They all chanced to be in the same stage, just before fusion of the two pronuclei, and are therefore the earliest embryos of the pig yet reported.

The ovaries and uteri of these animals are naturally of the greatest interest, and studies of them are now in progress.

SUMMARY

1. In the domestic sow, ovulation occurs during oestrus, probably on the first or second day.

2. Ovulation is independent of coitus.

3. The mature unfertilized ovum of the sow measures 155–165 micra in diameter, has a zona pellucida about 10 micra thick, and a yolk heavily laden with fat.

4. Fertilization of the ovum occurs in the Fallopian tube, as in other mammals.

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INTRA-UTERINE ABSORPTION OF OVA

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SEVEN FIGURES

While collecting embryological material for other purposes a decade since, my attention was not infrequently arrested by the presence of a degenerating or retarded sheep embryo in an apparently normal uterus the other horn of which contained an apparently normal foetus. Although these embryos were not infrequently quite normal in form they were always decidedly smaller than the normal embryos. In most cases the conditions suggested pathological changes both within the uterus and the embryo. The amniotic fluid was sometimes intensely turbid and even milky and more frequently dark in appearance and very evidently contained degenerating blood. Since twin pregnancies are not so very frequent in sheep, the number of cases seen was necessarily small. In uteri containing normal foetuses in the early stages of development, the embryo, the development of which had been arrested, was often represented by a rather firm fleshy mass of regular form which sometimes showed unmistakable evidences of degeneration even upon inspection with the unaided eye.

While engaged in the determination of the curve of prenatal growth in the guinea pig, Draper and myself found several cases of abnormal or at least regressive ova. All these abnormal guinea pig ova were much smaller than the normally developed ones of corresponding age should have been. Indeed, most of them were represented by firm oval fleshy masses, some of which possessed protuberances which made them look bicornuate. All were fastened with one end in more or less distinct uterine crypts which were especially evident in one of the smaller ova. The

instances met with so far were seen in pigs killed 19, 25 and 37 days after coitus.

In the first case that of guinea pig No. 16 only a single ovum was found present twenty-five days after coitus. This ovum was contained in the distal extremity of the right horn and was surrounded by a reddish black fluid. The uterus and adnexa appeared wholly normal to the naked eye. The ovum was composed of a slightly oval fleshy mass only 5 mm. in diameter although the normal embryo of this age measures 17 mm. It had a smooth regular surface and was still attached to the opened uterus but was easily detached. No placenta or foetal membranes were recognizable and the ovum protruded freely into the opened uterine cavity being attached to the uterine mucosa by its base with its longest diameter perpendicular to the latter. The line of attachment on the ovum apparently formed about one-eighth of its total perimeter.

On sectioning, the tissues of this ovum were found to be decidedly degenerated, the outer layers being composed of nothing but cell detritus. A little beneath the surface, this cell detritus is mixed with degenerating mesenchyme and variously-sized, better-preserved epithelioid cells. Between the latter lie large numbers of erythrocytes. These are scattered about freely and occupy other areas almost exclusively. Polykaryocytes and megakaryocytes in various stages of degeneration and different forms of leucocytes are also present. Some of the giant cells contain very bright golden pigment some of which is found also extra-cellularly. The framework of degenerating embryonic connective tissue, contains scattered cells and groups of cells with extremely large vesicular nuclei and prominent nucleoli.

Deeper beneath the surface remnants of blood vessels and of a reticulum which reminds one of that in young lymph nodes can be seen. In some areas, however, nothing but the degenerating reticulum with a little granular detritus remains. In addition to the giant cells large irregular masses which look like fused giant or other cells are also scattered through the specimen.

Sections made through the middle of the ovum show that the portion nearest the area of contact with the uterine mucosa is

best preserved and composed of a syncytium-like mass in which large vesicular nuclei predominate. This portion also contains a large vesicle lined by a low embryonic epithelioid syncytium. The spherical vesicle which in its largest portion comprises more than one half the diameter of the ovum contains nothing but a transparent fluid. Similar much smaller vesicles are also scattered about throughout the rest of this portion of the ovum some lying isolated at its very perimeter. A similar low epithelioid layer with indistinguishable cell boundaries also covers a portion of the surface of the most degenerated distal portion of the ovum where it also clothes villus-like extensions from the main mass. Some of the sections are almost surrounded by this epithelioid layer.

Small areas of these sections are practically devoid of tissue and contain almost nothing but a faint reticular network enclosing a slightly granular detritus and many polymorphonuclear leucocytes the nuclei of which have a typical horseshoe shape and the protoplasm of which is acidophile. Some of these leucocytes look decidedly degenerate and none seem to be phagocytic. In other often adjacent areas, the place of the polymorphonuclear leucocytes is taken by somewhat large cells with a vesicular nucleus which is circular in outline. The protoplasm of many of these cells is acidophile but here and there groups which look bright golden are seen. Most of these cells are well-preserved but some of them can be seen to be filled with similarly staining erythrocytes and what look like fragments and granules of erythrocytes.

Specimen No. 17 taken from a pig pregnant 19 days contained three ova, a normal one in the left horn and two abnormal ova in the right. The normal embryo weighed 35 mgm. and the smaller of the abnormal ova was approximately as large as the placenta and membranes of the normal embryo which weighed 0.91 mgm. The larger ovum was bicornuate. Both were single masses and no distinct placental portion was recognizable.

Both these ova which were no larger than a normal embryo of this age, were regular in form and their surfaces smooth. Upon microscopical examination, however, a few small, villus-like ex-

tensions were seen on the distal portion. A few small indentations were also evident but nothing else interrupted the regularity of the rest of the surfaces. The larger of these two ova was very well-preserved and much more vascular than that from pig No. 16. It was covered throughout by a low epithelioid syncytium which evidently was originally composed of a low cubical epithelium for here and there cell outlines are still faintly visible or the free surface of the syncytium is indented quite regularly so as to look crenated (fig. 1). These crenations are evidently the result of projections formed by the individual cells with the indentations located in the region of the former cell boundaries. The slightly irregularly-shaped, evenly-staining nuclei are thickly packed and although the cell boundaries are not clearly recognizable the layer is low and in places contains indistinct lines which look like remnants of cell boundaries and justify one in characterizing the cells as cuboidal. The rest of the ovum is composed of a syncytium containing large vesicular nuclei as shown in figure 2. Cell boundaries are distinct nowhere but the tissue apparently was a large-celled mesenchyme originally. Only a few small areas of almost complete degeneration are present. The tissue is densest and least vascular near the region of attachment to the uterine wall. The most rarefied tissue is found in the two cornua and near the distal portion where the loose mesenchyme contains small bloodvessels. Immediately beneath the investing epithelioid layer the specimen is completely canalized by wide capillaries which form an exceedingly vascular peripheral layer. A bit of the less vascular portion is shown in figure 3.

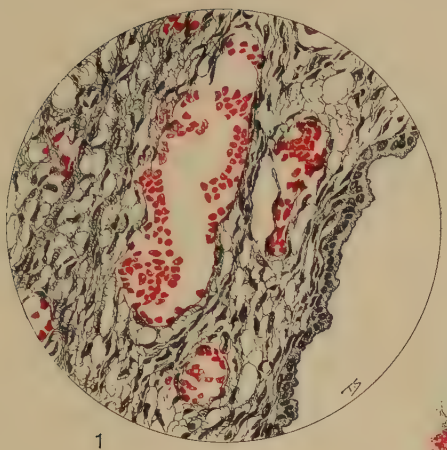
This specimen contains no large cavities but numerous smaller epithelioid-lined spaces are found in the cornua and the distal

Fig. 1 Structure of a portion of the periphery of a nineteen-day ovum. × 475.

Fig. 2 Structure of a portion of the periphery of another ovum of the same age. × 515

Fig. 3 Structure of the vascular portion of the ovum shown in figure 2. × 475

Fig. 4 Large nuclei from the central necrotic area of one of these ova. × 475



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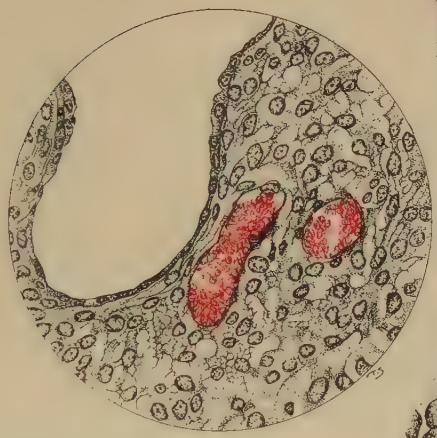

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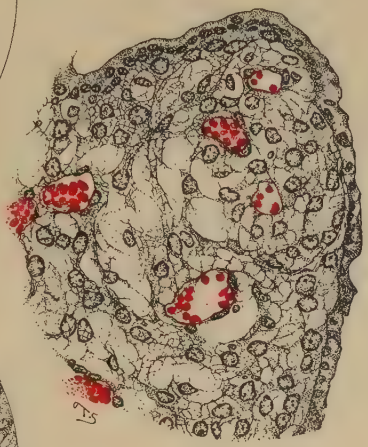

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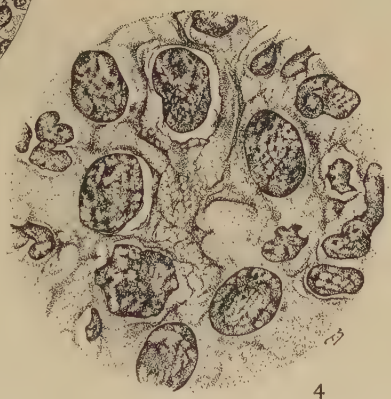

4

portions. The largest cavity which is lined by a rather low degenerated epithelioid layer is found in the center of the specimen but it is so small that it is not visible with the naked eye in the stained section. Although better preserved these cavities or cysts are similar to the very large one contained in the previous specimen. In spite of the condition existing in this specimen the blood is all contained in vessels only a few of which can be distinguished as veins or arteries. Some of the small arteries which are located in the basilar portion of the ovum near the uterine attachment have become completely obliterated. The portion of the ovum near the area of attachment is almost non-vascular as in the previous specimen. In some of the outer portions, however, and also near the placental attachment the tissue is of a more fibrous nature and looks far less embryonic. The blood cells are well-preserved and all the leucocytes have round vesicular nuclei in contrast to those found in specimen 16.

The second specimen from No. 17 was somewhat smaller and without cornua but it also was surrounded by an abundantly nucleated syncytium and was canalized beneath its surface by numerous capillaries as shown in figure 1. Portions of the surface were also pitted by crypts which gave the periphery of the sections a fenestrated appearance. All these crypts and vesicles are lined by a similar syncytial layer and all are empty. The specimen like the previous one is most vascular near the surface and near the fenestrated portion where the tissue composing it is also much looser. Only a few villus-like processes are seen in the distal portion.

Although half—apparently the proximal half—of this specimen was lost, the structure of the remaining half is practically the same as that of the previous ovum. Its preservation is not quite so good, however, for it contains partially necrotic and small liquefied areas in its interior. The more necrotic portions of this ovum contained nuclei truly gigantic in size. This will be evident on comparing the magnification of figure 4 with those in 1 and 3. It too is quite vascular and some of the vessels all of which are full of blood, are extremely large. Some portions of this ovum look more like fibrous mesenchyme others

more like sarcomatous tissue, but cell outlines are nowhere evident.

Specimen 19 in which the period of gestation was twenty-six days contained five abnormal ova, two in the left and three in the right horn. All of these were quite equally-sized, irregular masses but they were only about two-thirds as large as a normal placenta with that duration of pregnancy. They were easily detached and projected freely from the opened uterine cavity as had those in the previous cases. In all except one ovum the placental crypts were very shallow fossae but this specimen was contained in a definite funnel-formed uterine crypt about 3 by 3 mm. in size. Since these crypts were not noticed until the ova had been removed from the uterus I am inclined to think, however, that they were formed mainly by the post mortem contractions of the uterine musculature. This assumption is also suggested by the fact that all these ova completely fitted the lumen of the uterus and formed slight elevations on its surface. No placental portion was recognizable with the naked eye and there was no gross evidence of pathological changes in the uterus.

Although these five specimens of abnormal ova varied somewhat in size this variation was not marked. All were from 4 to 6 mm. long and 2 to 4 mm. thick and in contrast to the preceding specimens the four ova which were removed from the uterus had a dull fuzzy instead of a smooth shiny surface. They were exceedingly soft and rather irregular in shape. The contracted uterus which looked entirely normal was nodular in consequence of the enlargement opposite the ova. It contained no exudate and upon microscopical examination the ova were found well-preserved. All these specimens were but slightly vascular, the small capillaries being located mainly in the peripheral layers as before. They were all devoid of an outer epithelioid layer and were composed of a syncytium containing large nuclei none of which were nearly as large as those found in the preceding specimens, however.

As in the previous specimen the largest nuclei were found in the interior of the ova and the smallest at the surface where they were more elongated and where the syncytium took on a more

fibrous and stratified character because the tissues and the long axes of the nuclei, were arranged parallel to the surface. In one portion of one ovum the extremely large cells with their large oval nuclei are still preserved and give one a good idea of what the original structure of the ovum, in the early stages of degeneration really was.

No other type of cell was found except that the formation of the giant cell masses is indicated through coalescence of adjacent degenerating cells. The capillaries are engorged with erythrocytes and a few leucocytes with vesicular nuclei, but no vessels larger than capillaries of the ordinary calibre are present anywhere. The structure of these ova at the region of the uterine attachment corresponds to the rest.

One of these specimens still shows a little of an epithelioid covering in two very small places. In one of these the epithelium is shown in the form of a tube which may represent the remnant of a crypt or an invagination. Although this ovum is completely canalized by a plexus of fine capillaries near the periphery it contains no larger vessels in its interior.

One of these five abnormal ova from No. 19 was left *in situ* and cut serially in paraffine. It measured 5.5 mm. in diameter after fixation and completely filled the uterine cavity except in a few areas where small spaces were left between the ovum and the uterine wall. A cross section of the uterus and the contained ovum measured 6.5 mm. The musculature of the uterus was thickened nowhere, there were no indications of invasion of it by the tissues composing the fleshy mass nor was there any indication of cellular infiltration. Under low magnification the uterine mucosa was evident nowhere, however, and as seen in figure 5 there seemed to be no definite line of demarcation between the ovum and the surrounding uterine wall. The central portion of the fleshy mass was less dense and contained a relatively large irregularly-shaped degeneration cavity which contained what looked like a remnant of the embryo some portions of which were in direct contact with the surrounding tissue. The whole ovum was quite uniform in structure, however, though its vascularity varied somewhat.

Fig. 5 Uterine wall with a portion of the periphery of an ovum of twenty-five days. × 1340

Under higher magnification it was seen that glands of the uterine mucosa were present in a zone of loose mature connective tissue which probably took its origin from the submucosa and the inner portions of which were mingled with the tissues at the periphery of the ovum. The latter were also fibrous in character but much looser and more vascular and looked quite like normal embryonic tissues. They stained more with orange *G*,

however, while the maternal submucosa which contained some uterine glands stained deeply with fuchsin.

The outer portions of this ovum also, were composed of a loose fibrous connective tissue containing numerous capillaries filled with blood the erythrocytes of which were well-preserved. The thickness of this outer thin layer varied somewhat and from it to the center of the mass there was a gradual transition to large epithelioid cells which were plainly necrotic in places around the cavity which contained a remnant of the embryo. The latter was represented by very irregularly-shaped, folded hollow tubes composed of one and two layers of epithelioid cells which also showed signs of degeneration and apparently represent the ectoderm and entoderm. In one portion, shown in figure 6 *a–65* and *a–101* an indication of the mesoderm also seems to be present.

In some portions this embryonic tube was two-layered being composed of an outer layer of cubical and an inner of polygonal cells with large vesicular nuclei as shown in figure 6 *a–1*. In other portions the order of these layers seems to be the reverse. There were no evidences of phagocytosis and giant cells were not seen.

The next specimen was obtained from a guinea pig killed thirty-seven days after coitus. There were four fetuses, two in each horn. The distal one in the left horn was very evidently considerably smaller than the other three. The three large unopened apparently normal specimens weighed 12.2, 13.1 and 13.1 grams and the respective embryos measured 4.3, 4.5 and 4.4 mm. The fourth specimen which was abnormal weighed only 5.1 grams. A remnant of the foetus seemed to be contained in what looked like the greatly folded and collapsed membranes. The placental disc measured 1.5 cms. in diameter as compared to the normal ones which measured 2 cms. From these measurements and also from the weight of this intact specimen it is evident that the embryo in this ovum must have degenerated almost completely. This inference is also borne out by the microscopic examination. But the most interesting thing was the fact that abortion had not occurred.

Moreover, the fact that this specimen was contained not only in what appeared to be a perfectly normal uterus but was implanted within less than 1 cm. of a perfectly normally developed embryo is equally interesting and significant. The latter was as heavy and practically as large as the larger of the two embryos in the other horn. Huber '15 also found very young abnormal rat

Fig. 6 Sections from an embryo contained in the ovum a portion of which is shown in figure 5. The numbers under the illustrations represent the number of the section which was drawn. The sections were cut 10 μ thick. $\times$ 475

ova side by side with normal ones in apparently perfectly normal uteri. Although the abnormal ova found by Huber were very much younger than the specimen here recorded, the significance of the facts may be the same.

An incision made through this fixed specimen showed a necrotic hemorrhagic area in the center of the u-shaped mass of the

fixed placenta. Upon microscopic examination the most striking thing was the remarkable phagocytic activity in the center and the entire absence of comparable phenomena in the periphery of the placenta. There is an entire absence of phagocytosis and of hemorrhagic areas here although the nuclei in the mesenchyme are extremely large and degenerate.

It is as if absorption of this embryo and placenta were taking place from the interior of the specimen. The portion of the placenta directly beneath the embryo is decidedly hemorrhagic

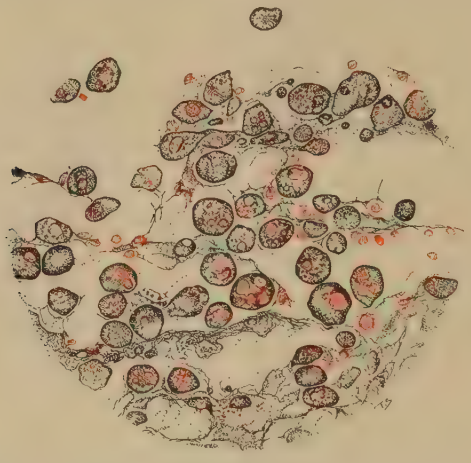

Fig. 7 A portion from the degenerating area of the placenta directly beneath the remnant of the ovum showing the remarkable number of macrophages. × 515

and very necrotic. Many of the large mononuclear phagocytic cells found in the central area and shown in figure 7, are so degenerate that they are mere shadows. Others are full of vacuoles and still others of erythrocytes and cell detritus. Many of them look so necrotic that one must doubt their ability to have remained actively phagocytic much longer even if they possess cell inclusions. The nuclei of the larger cells are usually small and not very evident but in the small cells which possess fewer or no cell inclusions they are relatively larger and are also stained better. Most of these macrophages have an acidophile

protoplasm. Although much blood is contained in this necrotic area only very few polymorphonuclear leucocytes are seen and these do not show evidence of phagocytic activity. In some areas these large phagocytes lie in a wide-meshed reticulum. For a discussion of the origin, relation and properties of these cells the reader is referred to Evans '15.

From figure 6 it is quite evident that although these ova may have developed normally up to a certain point they must subsequently have developed abnormally. The disproportion between the size of the embryo and the placenta alone shows this. Furthermore, since the very early rat ova described by Huber already showed degeneration phenomena it follows from this as well as from the relatively large size of the placenta that the life of the embryo must have been prolonged for a considerable period of time.

The cause of death of these ova must, to be sure, remain a matter of conjecture although the gross and microscopic character of the uteri would seem to indicate that the cause probably was intra- rather than extra-embryonic if it was not due to defect in the corpora lutea. At any rate death of the ovum did not seem to be due to a defective placental development although it must be borne in mind that it is possible even if not probable, that the uterine site upon which implantation occurred may nevertheless have been pathological. Such an assumption is made very unlikely, by both the apparently normal development of the placentae and by the surprisingly extensive development of the latter. The latter fact also seems to indicate that the early placenta even possesses considerable independence of the embryo.

Although the question as to whether or not abortion would finally have occurred in these cases must remain a matter for conjecture, I am ready to believe that such a termination would not have occurred. To be sure, such an assumption presupposes the gradual absorption of these ova, embryos and placentae and also raises the question as to what percentage of pregnancies terminate spontaneously in this way even after considerable development has occurred. If such a regression and

absorption occur in man also the surprising percentages of spontaneous terminations of pregnancies given by Mall '08 and '10 would be increased still further.

It will be recalled that Frankel '03 was able to cause death and intra-uterine absorption of ova in rabbits up to the twentieth day of pregnancy through destruction of the corpora lutea. Frankel found that abortion did not occur in these rabbits and described the gross changes as follows. The egg-chambers which became less tense because of a decrease in the production of amniotic fluid also became folded longitudinally, wrinkled and changed in color from a very red to a pale yellow. Frankel considered the reduction in the quantity of amniotic fluid as the first sign of regression and noted that the spherical chamber became more elongated, cylindrical, firmer and nodular. The embryo also became drier, smaller and more unrecognizable, finally being dissolved and represented only by an amorphous grayish white 'Schmiere.'

The placenta was preserved the longest and could be recognized as such for several days later. But it also became dry and pale red, only a few fragments finally remaining in the longitudinally folded and slightly swollen mesometrium. In this stage the uterus was only slightly firmer and showed but a minimal enlargement. After fourteen days even these evidences of a past pregnancy had disappeared only an anemic ring remaining and after three weeks not the least indication was left of the interrupted pregnancy.

Although the intra-uterine absorption of all guinea pig ova belonging to a single pregnancy could be due to defective development of the corpora lutea or entire lack of development of the latter it is much more difficult to see how regression and absorption of a single ovum lying between apparently normal ova could occur. It is conceivable, of course, that the growth of the corpora lutea might be sufficient for the development of four and not for six or more ova but one would expect all to suffer a corresponding retardation rather than have one or two destroyed and the rest preserved.

Since the ovaries of the guinea pigs concerned in these incidental observations were unfortunately not preserved I am unable to report on their condition. Nevertheless, even this small series of cases indicates that intra-uterine absorption of ova is not a rare phenomenon in guinea pigs. This conclusion is also in entire accord with Frankel's observation regarding rabbits. According to Koebner, Frankel concluded that the physiological regression of one chamber is very common, and emphasized that in many cases not all foetuses reach normal development. Individual fetuses die and are resorbed together with the placenta, while the rest go on to maturity. From these observations of Frankel which so far as I can learn were drawn from experimental work, Koebner observes that the rabbit is apparently less disposed to abortion in the first twenty days of pregnancy than to a "dry degeneration and resorption" of the fetus. Koebner '10 found that the bones also are absorbed under experimental conditions in the rabbit, and Williams '16 while discussing the subject of missed abortion in women states that "In very exceptional instances the entire product of conception may be absorbed without a sign of external discharge. Polano and L. Frankel have reported cases in which this occurred after the pregnancy had advanced as far as the fourth month and Koebner has demonstrated its possibility by animal experiments."

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METHODS OF MOUNTING SECTIONS IN GELATIN

J. B. JOHNSTON AND EDNA G. DYAR

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Gelatin has been used to some extent by various European workers for mounting sections, but has not come into general use. Our experiments show that gelatin possesses three properties which render it undesirable for this purpose until overcome by special treatment. Gelatin is non-hydroscopic, brittle and inelastic, and instable. In the extremely dry winter climate of Minnesota the gelatin dries and shrinks until it bends or breaks the glass slide on which it is spread, or cracks and peels off the slide. In very moist summer weather the gelatin may become whitish-opaque from moisture. When these qualities are corrected by the addition of glycerin to render the film hydroscopic, sugar to make it elastic and pliable and some hardening agent to render it stable, a satisfactory and permanent mounting medium is secured. To render the gelatin insoluble in water, formalin, chrome alum, chromic acid, tannic acid or potassium bichromate may be used with varying results. The most satisfactory product is obtained by means of potassium bichromate or chrome alum. After the addition of one of these salts the gelatin is rendered insoluble upon exposure to light. There is also produced a very slight grayish-green tint by the chrome alum and a deeper greenish tint by the bichromate, but this does no harm in thin films. Perhaps the best results are obtained by protecting the gelatin by means of an insulating varnish. The varnish seals the gelatin against the action of atmospheric vapor and also prevents the evaporation of water from the hardened gelatin film, the presence of water being necessary to maintain elasticity and pliability.

The advantages of a gelatin mounting medium are: saving of expense of dehydration for mounting in balsam and of the cost of cover glasses, and availability in some cases where an aqueous medium is necessary. It can be used on sections stained with haematoxylin, carmine and some but not all of the anilin dyes (e.g., not with acid stains soluble in water). Its use in films without glass may prove to have some value.

SOLUTIONS TO BE EMPLOYED

A. Best quality photogelatin 5 grams.
Distilled water 100 cc.
Add glycerin 5 cc. Let stand two hours.
Raise to 50°C. Gelatin dissolves.
Filter through canton flannel.

B. Hydrate 2 grams, gelatin in 45 cc. distilled water.
Add 10 cc. glycerin and 15 cc. corn syrup.
Raise to 50°C.
When gelatin is dissolved add 18 grams gelatin hydrated and dissolved in 55 cc. distilled water.
Filter through canton flannel.

C. Prepare as in B, using 2 grams gelatin and 45 cc. water and 23 grams gelatin and 55 cc. water.

D. To render A, B, or C insoluble add to the solution prepared as above 1½ cc. of a 10 per cent solution of potassium bichromate or chrome alum to every 100 cc. of the gelatin solution.
This solution must be used at once, since it will not melt after being allowed to harden.

In making the above solutions a temperature of 45° to 48°C. is necessary to dissolve the gelatin. After the gelatin is dissolved the solution may be lowered to 30°C. without causing the gelatin to set. In mounting sections it is necessary to keep the gelatin sufficiently warm to secure penetration, but advantage should be taken of the fact that the gelatin remains fluid at lower temperatures which are less likely to harm the tissues. In the following directions it is intended that the gelatin itself shall be kept at about the temperatures indicated, whether by means of an oven or a constant temperature plate.

For paraffin sections

Bring into distilled water on the slide. Place slide on constant temperature plate at 35°C. and cover with sufficient solution A to make a complete covering film when dry. After a few minutes on the warm plate set in a horizontal position to harden.

For celloidin sections up to a thickness of 100 microns

Carry the sections on paper ('onion skin' best). Clear in glycerin and water over night, followed by glycerin several hours.

Immerse sections in solution A in flat dish at 35°C., 20 minutes. Clean and flame[1] a slide and place on warm plate. Spread on slide a small amount of solution A and immediately place section on it, removing the paper.

Carefully press out all air bubbles.

Add more solution A and then drain to secure a thin but complete covering for the section.

Keep on level warm plate until gelatin is evenly spread.

Place in horizontal position to dry at room temperature.

[1] Flaming the slides is necessary to remove the thin film of organic material which is taken up from the air by slides exposed for any length of time. The usual cleaning solutions do not wholly remove this.

To secure a sufficiently thick and even covering, when the five per cent gelatin is used it is best to make the first coat thin and apply a second and third coat to the slide when cold, draining each time.

For thicker celloidin sections (1 to 2 mm.)

After clearing in glycerin immerse the sections for at least three hours in solution A at as low a temperature as practicable (30°C.) and mount in solution B by the method just given.

For mounting sections in films without glass

Fasten together two very thin celluloid films by means of snaps on one edge, place the section or sections between them and immerse in water or glycerin at 35°C. Drain, separate the films and sections as they are immersed in solution B in a flat dish on warm plate. Abundance of gelatin solution must be used, to secure complete immersion. After twenty minutes carefully press out the air bubbles with the fingers or a rubber wedge. Attach snap hangers to all four corners of the film and hang up to dry at room temperature. The hangers at the lower end serve as weights to prevent warping.

For thick sections (1 to 2 mm.) in films, immerse first in solution A at least three hours and then mount as just directed, using solution C. As this thick solution does not drain off readily, the excess gelatin should be removed from the surface by stripping the film between thumb and finger.

Solutions A, B, and C may have thymol added and be kept cold and remelted when wanted.

Finally, in each of the above cases either an insoluble preparation (solution D) should be used or the finished preparation should be covered with a varnish, as soon as it is dry enough not to be sticky. One may use the Zapon varnish found in the market. The same may be made by dissolving 5 grams celluloid in 20 cc. amyl acetate and 80 cc. acetone. A good varnish which is more pleasant to use is made by Abney as follows:

Alcohol 20 ounces
Ether 40 ounces
Pyroxylin or celloidin 400 grains

In applying the varnish care should be taken to have the gelatin completely covered. Films should be dipped in the varnish, and on slides the varnish should be spread with brush or spray beyond the edges of the gelatin.

December 8, 1916

THE VALUE OF ABSOLUTE ALCOHOL FOR REMOVING ADHERENT PARAFFIN SECTIONS FROM PAPER OR PASTEBOARD TRAYS

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Laboratory workers in embryology, histology, and neurology, and especially those whose work calls for the mounting of serial paraffin sections, have all at some time been annoyed by the adhesion of their sections to the paper or pasteboard tray on which they had been placed prior to mounting. This occurs, of course, most frequently during the warmer months of the year or where sections are not mounted for a long time; thus leading often to a complete loss of a valuable series.

Because of this it may be of value to call attention to the use of absolute alcohol as a means for overcoming this difficulty. The amount of alcohol used is very slight, being just sufficient to moisten the sections completely and in addition overrun on the sides so as partially to impregnate the paper or pasteboard to which they adhere. After this, several minutes time should be allowed for evaporation of the alcohol. This requires only a brief period, but can be hastened if a current of cool air is allowed to strike the tray. Where the sections adhere with especial firmness this last is particularly desirable.

It is of advantage when dealing with large sections to take a section-lifter or fine-bladed scalpel and run it along under the edge of the sections before the alcohol has completely evaporated. After this procedure sections which adhered firmly loosen almost always with ease.

The use of 95 per cent alcohol is not recommended since with it satisfactory results are less often obtained. It does not evaporate with the rapidity and thoroughness of the absolute alcohol. The latter vaporizes very readily and to this its action of loosening paraffin sections from paper or pasteboard trays may possibly be ascribed.

[1] Contribution No. 47, November 1, 1916.

PRESERVATION OF ANATOMIC DISSECTIONS WITH PERMANENT COLOR OF MUSCLES, VESSELS AND ORGANS

A SUPPLEMENTARY NOTE, DESCRIBING ANOTHER METHOD, THE CURING METHOD

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Since the printing of the original manuscript in the Anatomical Record, Vol. 10, No. 1, November, 1915, I have found out a new and simpler method, the curing method. It consists in the following: After the completion of the dissection, if the muscles present a dark brown color, the preparation is immersed in G33C1 for three days. G33C1 means a solution composed of 33 per cent of glycerine, 1 per cent of carbolic acid and 66 per cent of water. It registers 10° Baumé.

At the end of the three days it is taken out and exposed to the air in a room until the muscles become black. This requires about ten days. If some muscles do not blacken as fast as the others, they should be painted daily with pure glycerine until they become as black as the others.

As soon as all the muscles are black the preparation is immersed in Ch.F75, i.e., a solution of chloride with 75 per cent of formal, as previously described.

Then it is placed permanently in Ch.F5.

The results are quite satisfactory. The advantage of this method is that it does away with the use of calcium chloride, described previously, which is not always uniform.

Pale muscles do not do so well by this method. They should be painted in preference.

I have also brought out the following points which are of some assistance in the work.

In using tallow for distending the arteries, it is best to use beef tallow from around the kidneys. It is obtained from the butchers at the market. Tallow from corn fed beef is the best.

When the dissection is completed, if the muscles present a dark brown color they are suitable for the curing method. They are comparatively scarce. If the muscles show a lighter color than dark brown they are suitable for the paint method. They are much more common than the dark brown.

In the final preservation of curing preparations Ch.F5 will do as well as Ch.F.20; thus effecting a marked saving in the cost of the permanent solution.

I found that the following mixture of paint gives better results: Tuscan red, half teaspoonful; turpentine, 2 teaspoonfuls; lamp black, 2 grains. The lamp black tones down the Tuscan red when too bright in the solution. This quantitiy is more than enough to paint two or three times all the muscles of the upper extremity. Try the mixed paint on 1 or 2 inches of a muscle to see how the color will show and modify the paint accordingly.

When using lamp black, any dark red paint will do. Tuscan red is not so essential.

Two or three thin coats of paint are better than one thick coat. Thick coats make preparations look like daubs.

When the preparation is placed in the solution, if the color is not satisfactory, too light or too bright, do not hesitate to take it out, expose to the air for a few hours to dry and then repaint, with a suitably prepared paint. Do this two or three times if necessary. Final success depends on it.

Painted preparations do better decidedly on A10F.5 than in A10 alone. They do not require filtering as often and filter better. The F.5 prevents the bacterial cloudiness. It does not seem to affect the color of the paint any more than A10 or A20.

Artists' oil paints (Winsor and Newton) are better for the vessels than any other. Deep Vermilion for the arteries and Permanent Blue for the veins.

When the paint remains in the cup over night, even with a lid, it becomes too thick and turpentine must be added to it before using it.

A flat brush of fine bristles about $\frac{1}{3}$ inch wide is quite handy to paint large surfaces.

Keeping solutions clear all the time is the foundation of the preservation of the preparations.

When solutions have been filtered twice in succession, if they do not come out clear, they should be changed for fresh solutions.

Solutions very cloudy or very discolored filter badly. They clog the filters. It is best to make new fresh solutions.

When solutions get old (two or three years) they should be changed unless they remain clear.

THE DEVELOPMENT OF THE HUMAN CHIN

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Linneus is generally given the credit of having been the first to make the observation that the chin is a characteristically human trait In none of the anatomical or anthropological discussions of the chin has the writer seen credit for this shrewd observation given to any earlier scientist. Observation and record of the fact that the chin is a characteristically human trait is, as a matter of fact, centuries older than Linneus, and it is doubtful whether the latter did not copy it from some older authority. Pliny in Book XI, Chapter 60, of his Natural History, informs us that "no animal, with the exception of man, has either chin or cheek bones." The Greeks when in the act of supplication, touched the chin to show, as some would say, their affinity with the divine, and, if this is true, making fitting recognition of its human peculiarity as a trait not shared by the animals. But no Greek scientist seems to have speculated about its origin.

The attempt to explain the evolution of this anatomical feature is certainly comparatively recent. It appears to be no earlier than Cuvier. Cuvier remarks that in certain quadrupeds, individuals occasionally are born with the upper jaw unusually small; as a result the lower jaw, by the inturning of the alveolar processes to articulate with the unusually short upper ones, gives rise to a mental prominence bearing a close resemblance to the human chin. He claims to have seen an instance of this in a calf of Geneva, of reputed human paternity. Cuvier's explanation was a shrewd one. Sir Ray Lankester has used a precisely similar argument in accounting for the evolution of the chin of the elephant which, as he has shown, has developed pari passu

as the jaw has shortened, the unsupported upper lip meanwhile lengthening into the trunk of the modern elephant.

In the past century considerable attention has been given to the development of the chin and various explanations of its evolution have been offered. Any discussion of the problem should give some review of these views and we have attempted to summarize them below.

SPEECH

Attention has often been called to the effect, or supposed effect of speech upon the mandibular conformation—or vice versa Osborn, for example, reminds us that the narrow passage between the alveolar processes which, in the simia, lie in almost parallel, or, in some cases, in forwardly converging lines, give small range for the action of the tongue.[1] Whether this restriction of the play of the tongue would seriously interfere with speech, may be doubted. We speak of tongues wagging, but they do not really wig-wag so much as hump themselves while oscillating upward and downward in interfering with expiration. It is only upon failure of speech that we stick our tongues in our cheeks. The uplift in the human palate might be considered more favorable to speech than the increased width of the alveolar processes. Defects in the palate cause defects in speech, but we do not hear that people with long, narrow jaws have less linguistic facility than those with short, broad jaws.

The effect of the mandible upon speech has not, however, elicited so much defense as its converse, the effect of speech upon the mandible. In the mandible of simia will be found a deep pit lying in the inner concave surface. This seems to be absent generally in other mammals, including mankind. It accommodates the genio-glossus muscle which rises here and spreads out, fan-like, along the middle line of the lower surface of the tongue. This muscle, Dr. Louis Robinson believes[2] aids

[1] Men of the Old Stone Age, 100, 139–140, Scribners, 1915.

[2] Evolution of the chin. North American Review, Sept., 1914, vol. 200, p. 438–449.

the tongue in sorting out the contents of the mouth. The dog, for example, seems to have considerable difficulty in getting rid of undesired morsels, while cattle, the giraffe, and the camel, shift the undesired portions to one side, where long, projecting papillae help to work them out along the cheeks as they are agitated by the tongue. In place of this pit we find in the human jaw a bony prominence or tubercle, and Dr. Robinson professes to be able to trace the gradual evolution of this pit for the accommodation of the genio-glossus muscle, as we find it in the simia, to the bony prominence known as the genial tubercle which replaces it in the human jaw. The pit itself is subject to much variation, being most shallow in a fossil lemur, and shallow in the anthropoid apes, because a downward tilting of the margin of the jaw below the incisor teeth gives larger surface for the attachment of the muscle. The depression seems to be least in the siamang gibbon, while the human jaws of Heidelberg and Naulette are said to show it to a slightly less extent than the siamang gibbon. As these are among the oldest and most simian of human jaws the correspondence is not to be lightly passed over. Klaatsch[3] confirms the existence of a fossa sublingualis in the Heidelberg jaw, and says that in the Hauser skull from Le Moustier no genio-glossus spinal prominence is present, its place being taken, as also in the Mauer and Krapina G. skulls, by a fossa or pit for the insertion of this muscle.[4]

The insistence of Robinson is not without precedent. In 1904 Dr. C. Toldt, of Vienna, at a meeting of the German Anthropological Society of that city, suggested that the evolution of the chin was due to the development of the muscle emanating from the tongue and necessitated by speech. He returned to this argument the following year, in a paper entitled "Uber die Kinnknöchelchen und ihre Bedeutung für die Kinnbildung beim Menschen."[5] This paper called forth a reply by von Hause-

[3] Zeitschrift für Ethnologie, 41 ('09), 554–555.

[4] See also Hausemann, ib., p. 719, 721; R. R. Schmidt, Die Diluviale Vorzeit Deutschlands, 234 (Stuttgart, 1912); K. Gorjanovic-Kramberger, Der Diluviale Mensch, 176–181.

[5] Vienna Anthropologische Gesellschaft Metteilungen, 36 ('06), 51, 54.

mann, who insists that the ossicula mentalia are to be otherwise accounted for, and that other causes have been primarily responsible for the evolution of the chin.[6]

C. C. Blake[7] considered the absence of the genial tubercles in the La Naulette jaw purely adaptive: "The relative and absolute great thickness of the jaw at its symphysis originates this shelf-like structure, which is solely caused by the great deposit of osseous matter around the site of the genial tubercles."

Mr. Roosevelt finds the jaw of the chinless homo heidelbergensis so primitive that it must have made his speech thick and imperfect.[8] The veteran French anthropologist, Paul Topinard, is not impressed by such arguments. In his discussion of the significance of the absence of the genial tubercles in the La Naulette jaw[9] he points out that only a small portion of the muscles from the tongue, namely, the geniohyoid muscle, which reaches from the small hyoid bone at the base of the tongue to the symphysis, is attached to the lower part of the tubercles, the genio-glossus muscle finding attachment to the region above this. Instead of accepting the presence of tubercles as an advantage, he insists that the depression in the gorilla's mandible gives him considerable advantage over man. Albrecht found the mandible of an idiot of twenty-one years of age possessing tubercles that attained the extraordinary eminence of 9 mm.[10] This outvies the tubercles of educated orators. This idiot, observes Topinard, did not have half the persuasive eloquence doubtless enjoyed by the La Naulette lady.

[6] See his paper on Die Bedeutung der Ossicula Mentalia fur die Kinnbildung. Zeitsch f. Ethnol., 41 ('09), 714–721; see also P. Bartels in Int. Monatschre f. Anat. and Phys., vol. 21, p. 179 ff.; Osborn, op. cit., 228; R. Virchow, in Zeitsch f. Ethnologie, 14 ('82), 287; Schaaffhausen, in Korr. d. Deutsch Gesse f. Anthrop. (Jan. 1881), No. 1, p. 3; L'Anthropologie, 4 ('93), 753–754.

[7] Anthropological Review ('67), vol. 5, 295–302.

[8] National Geographic Magazine, Feb., 1916.

[9] Rev. d'Anthropologie ('86), serie 3, vol. i, 416–25.

[10] See Bull. Soc. Anthrop. d. Bruxelles, i ('82–'83).

DECREASE IN THE SIZE OF TEETH AND OF THE ALVEOLAR PROCESSES

When our forebears assumed the erect posture and were able to fight freely with those fists which no longer had to serve as supports when running, the dangerous canines became shorter, and there was a gradual degeneration in the size, if not in the number also, of the teeth. The large bony alveolar processes in which they lay embedded were no longer needed, and there was a corresponding shrinkage in this bony structure. The absorption of the alveolar region leaves the lower portion of the mandible, which is more solid and less liable to change, relatively prominent and suggestive of a chin.

This view has been popular. Toldt, in the paper referred to, insists that the reduction in the size of the teeth, together with the drawing in of the enfolding alveolar processes, would tend to develop the chin. His commentator, von Hausemann[6] recognizes the force of this portion of Toldt's argument. Bardeleben[11] pointed out that the building up of the chin would result from reduction in the size of the teeth, and declared he knew of no exception to this correlation. In accounting for the reduction, he not inaptly likened the building of the chin to a hillock left out-standing on a plain where erosion has reduced the general level. The osseous portion which is left outstanding, like this older stone formation, becomes a protruding chin. The receding of the lower jaw is not to be forgotten. In this recession the teeth and the alveolar processes are especially involved, so that the protuberantia mentalis gradually comes to the fore—as we find even with prehistoric man. Similarly, Arthur Keith, while attributing to the muscles of the tongue a tendency to give a forward development to the chin, lays more stress on the recession of the alveolar processes that accompanies reduction in the size of the teeth.[12]

Robert Munro in 1912[13] attributed the prominence of the chin to retrocession of the facial bones, "as the shortening of

[11] Anatomischer Anz., 26 ('05), 107.

[12] Man: A History of the Human Body, 193–194. No date.

[13] Paleolithic Man, 19 (Macmillan '12).

the alveolar ridges would cause the teeth to assume a more upright setting in their sockets." Walkhoff noted the tendency of the reduction in teeth and alveolar processes to give rise to a chin.[14] Weidenreich[15] viewed the progressive development of the chin as purely a passive process: it got ahead by remaining where it was, the superior alveolar region being meanwhile in retreat. Rudolf Martin is inclined to champion these views[16] as is also Osborn. The latter adds to this tendency the growth and specialization of the muscles of the jaw and tongue employed in speech, though he insists that absence of the chin does not betoken inability to speak. Prof. T. T. Waterman has recently added his support to this school.[17] That absorption of the alveolar processes will leave the chin prominent is proved in the changes that take place in old age, where both process and result can be observed.[18]

Thus the changes that take place during the life of the individual are to some extent an epitome of those that are recorded by prehistoric evidence. "In all modern races of men the front part of the semicircle arch of teeth has shrunk or 'withdrawn' considerably, or more than has the bony jaw in which the teeth are set. Consequently the bone projects in front of the teeth as the bony chin."[19] Robinson recognizes the argument that shrinkage of the alveolar processes gives rise to the chin, but discountenances it.

[14] Die menschliche Sprache in ihrer Bedeutung für die funktionelle Gestalt des Unterkiefers. Anat. Anzieg., 24 ('03), 129–139; Beitrag zur Lehre der menschlichen Kinnbildung, ib., 25 ('04) 147–160; see L'Anthropologie, vol. 15, 99–100, 235–236 ('04). Similarly Frizzi, Archiv. f. Anthrop, ('10), vol. 37, p. 255 ff.

[15] Die Bildung des Kinnes und seine ungebliche Beziehung zur Sprache. Anat. Anzeig., 24 ('03–'04), 545–555.

[16] Lehrbuch d. Anthropologie, 873–874, Jena, 1914.

[17] The evolution of the chin. American Naturalist, April, 1916.

[18] See the description and illustration E. G. Norris, Human Anatomy, 65. Much valuable material will be found in the article by Ernst Frizzi, Untersuchungen am menschlichen Unterkiefer mit spezieller Berücksichtigung der Regio mentalis. Archiv. fur Anthropologie, 37 ('10), 252–286. Contains extensive bibliography, 101 sketches, and detailed measurements of 100 mandibles of different races.

[19] E. Ray Lankester, Diversions of a Naturalist, 250–252 (London 1915).

OTHER OSSEOUS CHANGES

The changes in the anterior portion of the mandible, if we compare that of an anthropoid with that of man, are not exhausted in the differences already noted, but include other important features. The os mentale in the simia is narrow, regular in curvature, and of smooth surface. The human os mentale often has a jagged border, and may be medially concave both in a horizontal and in a vertical plane, when viewed anteriorly. Most striking of all, it possesses, on both the right and the left side, a small bony protuberance or ossicle, which gives roughness to the contour, adds to the impression of greater breadth, and breaks up the convex lines into horizontal concave surfaces. Where normal osseous changes are not located at articular surfaces, we usually have to account for them as due, either to some shift in adjacent bony tissue which directly affects them, or to the action of muscles. The importance of the tuberosities at the symphysis in giving prominence to the chin, should not be forgotten. Their importance as a factor in the development of the chin has been recognized by B. Adachi.[20] Mies points out the value of the ossicula mentalia in building up the chin, and also the fact that paired muscles are associated with paired ossicles, whereas an undifferentiated muscle is associated with the undifferentiated ossicle.[21] A similar argument was later adduced by Toldt, who attempts to show that the development of the ossicula mentalia, which proceeds along different lines in the human and in the simia, has been influential in giving rise to the chin.[22]

MUSCULAR AND MECHANICAL FORCES

That these bony protuberances are directly related to muscle development is more than probable. Bardeleben's extensive investigations show that man alone possess on the os mentale

[20] Über die Knöchelchen in der Symphyse des Unterkiefers, Zeitsch. f. Morph. und Anthrop., ('04) 7, 369–372.

[21] Über die Knöchelchen in der Symphyse des Unterkiefers vom neugeborenen Menschen. Anat. Anzeig., ('93), 8 p. 361–356.

[22] Die Ossicula mentalia und ihre Bedeutung für die Bildung des menschlichen Kinnes. Sitz. d. Kaiser. v. Akad. d. Wiss. ('05), 114, (AB. 111), 657–92.

the paired (paariger) muscle, the musculus anomalus menti, which is associated, whether as cause or effect, with this bifurcation of the os mentale in the human. Not only is there this difference between simia and homo sapiens, but, as a consultation of G. Rugge's, Die Gesichtsmuskulatur der Primaten[23] will show, considerable differences in the muscular system of the two are represented in this portion of the face.

If the hand is held on the collar bone, the chin tilted in air, and the skin covering the chin raised as far as possible, the hand will detect movements over the surface of the clavicle. These movements are effected by a large muscle, or system of muscles, which rises in the lower lip, passes over the os mentale, down the front of the neck, and over the clavicle and some of the upper ribs. In the prosimia a large bundle of platysma pass over the os mentale, due partly to the narrowness of the anterior margin, partly to the demand for larger muscles in the larger lips which do much more heavy work than the human lips. The tendency wherever muscles pass over a bony surface and pull against it, is to flatten that surface. Hence the regular rounded contour of the os mentale in the simia, with absence of outstanding bony prominences such as we find in the human mandible.

There is, moreover, in the human, a specialization of muscular development which has proceeded far beyond that of the apes. The os mentale is traversed also by a set of muscles known as the musculus mentalis, which run at almost right angles to the platysma. In the human face these systems are separate and specialized, while in the simia they are often so interwoven as to make it impossible to entirely separate them. In Ateles, for example, it is difficult to distinguish parts of the mentalis from the platysma, while in the chimpanzee the complication is even more marked. Similarly, according to Robinson, there is both greater development and greater specialization in the genio-glossus muscle in human beings then is to be found in the apes. "In man the genio-glossus has become a series of a large number of independent muscular strips which are to all intents and purposes separate muscles, each with its little fiber of the hypo-

[23] Leipzig, 1887.

glossal nerve entering it in such a way as not to hamper its free movement, while in the apes it is apparently a single muscle, or a closely united group, acting en bloc."[24]

It has been already mentioned that the paired muscle on the os mentale is found only in human beings, and that it seems to be related to the external genial tuberosities peculiar to the human os mentale. Rugge noticed that the mental region of the human skull is distinguished from that of the simia by the possession of numerous tubercles which must be supposed to be the points of origin for many small muscles not to be found in the apes.

We need only consider the comparative range and facility of facial expression in the two species to grasp both the fact and the explanation of the existence of these numerous tubercles that cover the human mandible and their absence in the simia. The rapid pull of facial muscles used in articulation may have contributed not a little to this result. Some of the muscles used in such facial twitchings as laughter, for example, involves, find attachment on the anterior surface of the mandible. Here the bony prominences that rise to give these muscles attachment help in the outer construction of the chin. No one who has read the detailed account of the muscles used in facial expression, given by Prof. Arthur Thomson in his Anatomy for Art Students, can be skeptical about the much greater specialization of facial muscles in the human being and the tendency of these to elicit bony prominences on the skull and the mandible as points for attachment.[25]

It is not improbable that this difference in muscular pull will do much to explain the simian type of chin. The thick-lipped peoples, as notably the Negroes, have, of course, larger muscles to work their larger lips, and they have less prominent chins. Those negroid peoples who have thinner lips have more prominent chins. The chin is well developed in the Eskimo, though they possess a long and heavy mandible, and large teeth. In the typical negro the chin is but feebly developed, in keeping with

[24] An. Rep. Smith. Inst., 1914, p. 603; Knowledge (London), Nov., 1913.
[25] See also Alfred Fripp, Human Anatomy for Art Students.

the heavy, thick, protruding lips, though the negro mandible is not larger, longer, nor heavier, nor are the teeth larger than those of the Eskimo. In the more orthognathous Bushman, with smaller, thinner lips, we find, on the contrary, a well developed chin of the anteriorly concave type. There is, in fact, a general, though not a complete, correlation between thick, prominent lips and retreating chins.

The protruding teeth which are associated with the retreating chin, enable the animal to get rid of the food with comparative ease. In fact, if the teeth were set upright, as are ours, to disgorge would be fraught with considerable difficulty. If this advantage of being able to get rid of unwelcome and retain desired contents is to be preserved, large, long, strong upper lips are needed in a land where many hungry mouths linger round to pick up a fallen morsel or snatch a disappearing one. Our remote ancestor, 'probably arboreal,' had to keep a tight under lip until he could carry his head as high as the descendant who became lord of all he surveyed. Then large under lips were no longer necessary. In our contemporaries they serve merely to proclaim the proximity of their simian ancestry.

Mr. G. F. Scott Elliot asserts that in consequence of the broadening of the skull and the shortening of the face into a shorter, rounded-arch shape, great strain and cross-tensions would be thrown on the extreme forward points of the jaw-bones.[26] In this I do not follow him, for it seems to me the tendency would, if anything, be the opposite. Nor do I follow him in his suggestion that if the origin of bone-forming tissue may be due to muscular stresses and strains, then the production of bone-forming tissue at the chin would be favored—unless he means such stresses and strains as we have indicated. So far as mastication is concerned, the muscular stress would be much greater in the simia, which use their heavy, protruding lips in lieu of free hands, to pull in their food, and certainly when eating, use them much more than we use ours. But the masticatory muscles are not attached to the chin and could scarcely affect the os mentale directly. Simia must do more

[26] Prehistoric Man and His Story, 76–77; (London, 1915).

severe work with canines and incisors than we do with ours, but the muscular strain comes much further back on the mandible than Elliot supposes.[27]

The muscles used in deglutition must, of necessity, be larger and stronger in the simia than in human beings, and, so far as they attach to the anterior part of the mandible, we might expect them to exert some influence on its conformation. That these muscles do exert an influence on the shape of the chin is more than probable. Bijvoet, in a detailed study of the morphology of the musculus digestricus mandibulae in the zoological world, including man and the primates, demonstrates its varying area and method of attachment, which is not the same in those animals which chew with a scissor-like motion in the vertical plane, as in those whose jaws move with a side to side grinding motion—differences which are to some extent typified in man and the monkey, Duckworth's assurance to the contrary notwithstanding.[28]

CHANGES IN HEAD FORM

The lower jaw is not anatomically a part of the skull, yet it would be wrong to suppose that we can consider it as a feature isolated from the remainder of the head, since, physiologically, it is an integral part of the head. The upper alveolar processes are the supplementary portions which make, with the mandible, a functional unit. The various portions of the skull are so closely interrelated, either structurally or functionally, that any considerable change in a given region is apt to be reflected by

[27] The method of muscle attachment has been well shown by C. Toldt, Der Winkelfortsatz des Unterkiefers beim Menschen und bei den Säugetieren und die Beziehungen der Kaumuskeln zu demselben. Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften, 1905, vol. 114, (Abteilung III), 315–478. Further descriptive and illustrative account of the differences between the human and the simian will be found in C. Toldt's papers, Der vordere Bauch des M. digastricus mandibulae und seine Varietäten beim Menschen; and, Der digastricus und die Muskeln des Mundhöhlenbodens beim Orang.; Sitzungsberichte der Kais. Acad. d. Wiss., 1907. Ab. III, vol. 116, p. 373–459; vol. 117 ('08) p. 229–324.)

[28] Bijvoet, Zeitsch f. Morph. und Anthrop., 61 ('07–08), 249–315. W. H. L. Duckworth, Anthropology and Morphology, vol. I ('15).

corresponding or compensating changes throughout the entire skull.[29]

The upper alveolar region is an incorporated portion of the facial skeleton, and is dependent for its evolution upon changes in the facial region. The face is, in turn, architecturally but a pendant portion of the upper supporting skull case, so that changes in the skull case, affecting the points of juncture with the facial bones, facilitate changes in the facial proportions and with these the conformation of the alveolar regions. Dr. Karl Gorjanovic-Kramberger insists that an intensive construction of the chin is possible only through a change in the angle of prognathism, and reduction in length and size of teeth. The change in the angle of prognathism throws the roots of the front teeth to the fore and bony tissue must be built up there to give them strength. With this change in the angle of the teeth the mental prominence becomes more pronounced and the construction of the chin is assured.[30] He alleges also, and with some show of reason, that the construction of the chin in modern man has been directly correlated with the arching of the prognathous mandible. Toldt, similarly, has attributed the formation of the chin to progressive changes accompanying the widening of the face and the arching of the mandible.[31]

The changes that have taken place with the assumption of the erect posture and the use of softer food, have been fairly uniform, and are seen in many portions of the skull. As the

[29] This has been ably demonstrated by Mr. Francis Knowles in his study of the correlation between the interorbital width and other measures and indices of the human skull. Journ. of the Roy. Anth. Inst., 41 ('11), 318–49. Herman Welcker, in an article on Die Zugehörigkeit eines Unterkiefers zu einem bestimmten Schädel, etc. (Archiv. f. Anthropologie 27 ('01–'02), 37–106, has ably demonstrated the interdependence of the mandible and the skull. See also Aurel Von Török, Über Variationen und Correlationen der Verhältnisse am Unterkiefer; Zeitsch f. Ethnol, 30 ('98), 125–182. The correlation is, of course, not absolute. Exceptions exist, but the reference to them as 'disharmonic' types is evidence of the rule.

[30] See the section on Zur Bildung des Kinnes beim Homo primigenius, in the author's Der Diluviale Mensch von Krapina in Kroatien, 171–176, (Wiesbaden, 1900).

[31] Corresp. Blatt. d. Deutsch Gesells fur Anthrop., 35, ('04), 94; see L'Anthropologie, vol. 16, p. 583–584.

foramen magnum moves forward and the head becomes better balanced, less muscular pull is required to keep it in position. The mastoid muscles degenerate, pressure along the temporal bones decreases, the forehead emerges, and the head increases in breadth. The face, at the same time, broadens, because its foundation walls in the calvarium have begun to shift laterally. The alveolar processes must shift laterally also. This lateral spread of the palatal region arches the frontal portion of the alveolar region, which tends to be pointed or straight in the simia, and draws the incisors in, so that they no longer project, as hitherto. The face projects less, and with the straighter face the teeth, which in both simia and homo sapiens follow the facial angle, approximate the vertical; for the teeth must conform to the plane of the bony tissue in which they are set. Since the mandible is useful only as a correlative portion of the facial structure, it must conform to the changes effected in the skull. In doing so the chin becomes, of necessity, more and more prominent.

In the transition from the simian to the human type, we must take into account also the change that has come about in the articulation of the incisors. In the simia the incisors meet. This is not uncommon in palaeolithic man and in some of the more prognathous people. Among ourselves it still occurs, but only in a small percentage of cases. In the gorilla, indeed, the upper canines actually overlap the lower to a marked degree, so that the diastema on the lower alveolar region is much more marked than that on the upper. Already, then, the relative prominence of upper and lower incisors seems secured by this forward grasp of the upper canines. Man is standing proof of the triumph of the facial canines over the mandibular, for in homo sapiens all of the upper incisors, as well as the canines, easily overlap the lower, though sometimes the reverse occurs. It must, moreover, be borne in mind that the anatomical independence of the mandible, even where there is no physiological independence, allows the lower alveolar processes to accommodate themselves more rapidly to the new conditions, and we might expect that they would be more rapidly influenced by changes

in diet than would those of the palatal region. This would account for their more rapid retreat, while at the same time the enclosing upper incisors help by means of pressure from without.

We do, as a matter of fact, find that the changes in the mandible have gone further than those in the facial portions. In primitive man the alveolar processes are still at the outer margin of the lower facial region. But not so in the jaw of civilized man with prominent chin, where the alveolar processes lie behind the underlying heavy bony tissue, so that the teeth no longer conform to the plane of the bony tissue in which they are embedded. We do not find such considerable changes in the facial portion as in the mandibular.

We conclude, then, that no one factor should be singled out and given the credit for having evolved the chin, but that many forces have contributed to this result. Widening of the dental arcade gives more play for the tongue, whether for shifting food or consonants. It makes a better masticator, and perhaps, a better linguist. The interrelation may be close. Which is cause and which effect may be difficult to determine, for we have, not one, but many, parallel or interrelated developments converging in that peculiar human feature, the chin, which muscular forces, both within and without. have helped to design.

NOTES ON TWO CASES OF ANOMALOUS RIGHT SUBCLAVIAN ARTERY

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Cases of anomalous right subclavian have been reported occasionally, but their rarity and interest from an embryological point of view, besides their possible clinical relations, warrant their addition to the anatomical literature. Holzapfel ('99) collected two hundred cases of abnormal right subclavian artery including four of his own, and discussed them from anatomical, developmental, and practical standpoints. He gave the frequency as one case to one hundred sixty-seven cadavers, or .6 per cent. Cobey ('14) gives the results of ani nvestigation for the Anatomical Society of Great Britain and Ireland as five to five hundred, or 1 per cent. The cases here reported are two to two hundred thirty-seven cadavers, or 0.8 per cent. Bean ('04) reported two cases of his own and six others in the literature besides Holzapfel's cases. Cobey and Bevier ('15) have reported each one case.

Case 1. The subject is a white male, age sixty years. The arcus aortae gives off three branches, the truncus bicaroticus, arteria subclavia sinistra, and arteria subclavia dextra, the anomalous branch. It extends in a gentle curve upwards, backwards, and to the right from the level of the lower border of the first left costal cartilage to the left side of the body of the third thoracic vertebra. This abnormal course of the arcus aortae is produced by the rotation of the base of the heart towards the left. The aorta ascendens, therefore, lies ventrad and to the left of the aorta descendens, both lying to the left of the vertebral column. The truncus bicaroticus is flattened ventrodorsally, and, of course, lies to the left of the trachea and the midline. The A. subclavia sinistra is the second branch. The third branch is the anomalous A. subclavia dextra arising from the right side of the aorta descendens at its commencement. It crosses the vertebral column behind the oesophagus and trachea, diagonally cephalad and to the right, lying on the body of the third thoracic vertebra. It is considerably dilated up to the point where it emerges from behind the oesophagus. The great veins are normal. The trachea is normal. The oesophagus shows an interesting abnormality by making a detour to the right for a distance of 10 mm. where the anomalous A. subclavia dextra emerges dorsad to it. The right vagus preserves its usual course in the thorax. The N. recurrens is absent, the cardiac branch

of the vagus, usually arising from the recurrens, being supplied directly from the vagus trunk. Three or four twigs, taking the place of the recurrens, pass from the nerve trunk directly to the larynx. The left vagus is normal in its course and branches. The N. recurrens, however, passes across the ventral aspect of the anomalous subclavian. The ductus thoracicus is normal in its course. The phrenic nerves are normal.

Case 2. The subject is a white male of advanced years. The arrangement of the arcus aortae and its branches is similar to that described in Case 1. However, the truncus bicaroticus is cylindrical, and the abnormal A. subclavia dextra is not dilated.

In Holzapfel's cases 33 were dilated out of 51 in which that feature of the abnormal artery was discussed, or 64 per cent. One of the cases collected by Bean was dilated. The clinical effects of a dilated abnormal artery are to be taken into account, therefore. Holzapfel discusses this point and concludes that only by an aneurysmal enlargement of the artery may dysphagia lusoria be considered.

Surgically the abnormal course of the artery is important in attempts at ligation; in one instance reported it offered a decided difficulty. In surgical conditions involving the oesophagus or in operations on the thorax its presence may be of considerable consequence. The internist should be interested in the possibility of its causing unequal radial pulsations. Holzapfel believes it is not the anatomical cause of left-handedness, although it is to be noted that the two conditions exist in several cases. Cobey suggests the possibility of the abnormal artery producing symptoms similar to those of cervical rib, with resulting trophic changes in the extremity.

LITERATURE

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